Testing the suitability of harpacticoid copepods as food for marine fish larvae

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Content

Summary

While capture fisheries have been stagnating since the 1990's, aquaculture production has increased steadily from being negligible in the 1980's to sharing now 40% of the world fish supply. This increase entails a growing demand of fingerlings which results in an intensification in rearing fish larvae. Intensive rearing systems imply exogenous feeding which in turn led to a research activity to find food for fish larvae which is nutritious and can be produced cost effectively, since food is the highest cost factor in aquaculture production.

Brachionus and *Artemia* are nowadays the normally used prey items for fish larvae. They can be produced in high densities with relatively low labour costs. However, they have to be enriched, e.g. with certain fatty acids, to fulfil the nutritional requirements of fish larvae and are still outperformed by copepods which are the natural food of marine fish larvae. Fish larvae fed with copepods show higher survival and growth rates as well as fewer malpigmentation and malformations. For this reason, this study focused on the rearing of copepods, especially harpacticoids as food for marine fish larvae. Harpacticoids can be reared in higher densities than calanoids and are more robust to salinity and temperature changes, which promise an easier and a more cost effective production.

A copepod screening revealed *Tachidius discipes* as a possible new species as food for marine fish larvae. In the first chapter the effect of algal species on the growth performance, reproduction and fatty acid composition of *T. discipes* was investigated and compared to *Tisbe* sp. Additionally, the food saturation density was determined to allow an economical feeding procedure of the copepods. The two algal species *Rhodomonas* sp. and *Phaeodactylum tricornutum* revealed the best performance of both copepod species meeting the recommended ratio of essential fatty acids. *T. discipes* can compete with *Tisbe* sp. in terms of development time and reproduction when feeding on a nutritious food source, but *Tisbe* sp. had a higher fatty acid desaturation capacity and/or is a more opportunistic species which can compensate an inadequate food source by switching to other sources such as bacteria compared to *T. discipes*.

Nevertheless, *T. discipes* was evaluated as a new food source for Baltic herring larvae (*Clupea harengus*) and compared with *Brachionus plicatilis* (Chapter 2). A surprisingly low performance of the herring larvae fed with *T. discipes* led to an in-vitro trypsin digestibility test of several prey types used in aquaculture. This study revealed a lower digestibility of *T. discipes* than *Tisbe* sp. The most digestible prey item was *Artemia* sp.

Summary

Subsequently, a 2D-video analysis was conducted to investigate if the benthic living mode of harpacticoid copepods is posing a problem for pelagic fish larvae in terms of feeding success and energy demand (Chapter 3). Benthic copepods are obviously detected by pelagic fish larvae, but the larvae had a lower feeding success when feeding on copepods compared to *B. plicatilis*, especially at first feeding. However, this improved with ontogeny. Providing harpacticoid copepods via a floating sieve improved the feeding success and lowered presumably the energetic expenditure of fish larvae.

In conclusion, harpacticoid copepods can be a valuable food source for marine fish larvae, but their digestibility is a critical point, which should be considered when evaluating new species and conducting further feeding experiments. Furthermore, although harpacticoid copepods are cultured in relatively high densities, they do not reach the densities obtained with *B. plicatilis*. Consequently, copepods will be a food supplement rather than the sole food source.

Zusammenfassung

Während der Fischfang seit den 90er Jahren stagniert, steigt die Aquakulturproduktion stetig an. War die Fischzucht in den 80er Jahren nur in geringem Maße vorhanden, so nimmt sie jetzt 40% der weltweiten Fischversorgung ein. Diese Zunahme brachte auch einen steigenden Bedarf an Setzlingen mit sich, der eine Intensivierung der Fischlarvenzucht notwendig machte. Intensive Aquakultursysteme benötigen eine Fütterung von außen. Dadurch erhöht sich die Forschungsaktivität, Futter zu finden, welches nahrhaft ist, aber auch kosteneffizient produziert werden kann.

Brachionus und *Artemia* sind momentan die zwei meist genutzten Futterarten für Fischlarven. Sie können zwar mit geringem Arbeitsaufwand kultiviert werden, aber sie müssen mit Nährstoffen - insbesondere Fettsäuren - angereichert werden, um die Fischlarven adäquat zu versorgen. Für marine Fischlarven sind Copepoden ein Hauptbestandteil der natürlichen Nahrungsquelle. Werden Fischlarven mit diesen gefüttert, zeigen sie oftmals eine höhere Überlebensrate, ein besseres Wachstum und weniger Fehlpigmentierungen und Missbildungen im Vergleich zur Ernährung mit angereicherten *Brachionus* und *Artemia*. Aus diesem Grund liegt der Fokus dieser Studie auf der Zucht von Copepoden, insbesondere harpacticiden Copepoden als Futter mariner Fischlarven. Harpacticide Copepoden können in höheren Dichten als calanide kultiviert werden und sind robuster gegenüber Temperatur- und Salinitätsschwankungen. Dies verspricht eine leichtere und kosteneffizientere Zucht im Vergleich zu calaniden Copepoden.

In einem Copepoden-Screening erwies sich *Tachidius discipes* als ein möglicher neuer Kandidat zur Aufzucht von Fischlarven. Im ersten Kapitel wurde die Eignung der neuen Art im Hinblick auf Wachstum, Reproduktion und Fettsäurezusammensetzung untersucht und mit *Tisbe* sp. verglichen. Außerdem wurde die Futtersättigungskonzentration bestimmt, um eine ökonomische Fütterung der Copepoden zu ermöglichen. Mit den beiden Algenarten *Rhodomonas* sp. und *Phaeodactylum tricornutum* zeigten die Copepoden die beste Performance und das empfohlene Verhältnis der essentiellen Fettsäuren wurde erreicht. *T. discipes* und *Tisbe* sp. wiesen gleiche Wachstums- und Reproduktionsraten auf bei Fütterung mit optimalem Futter. Aber *Tisbe* sp. hatte anscheinend eine höhere Kapazität Fettsäuren zu desaturieren und/oder es ist eine opportunistischere Art, die inadäquates Futter kompensieren kann, indem sie zu anderen Futterarten wie z.B. Bakterien wechselt.

Dennoch wurde *T. discipes* als eine neue Futterart für Heringslarven (*Clupea harengus*) evaluiert und mit *Brachionus plicatilis* verglichen (Kapitel 2). Eine

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überraschend schlechte Konstitution der Heringslarven bei Fütterung mit *T. discipes* führte zu einem anschließenden In-vitro-Verdauungstest mittels des Enzyms Trypsin. Diese Untersuchung zeigte, dass *T. discipes* schlechter verdaulich ist als *Tisbe* sp. Eine noch weitaus höhere Verdaubarkeit wies *Artemia* sp. auf.

Anschließend wurde eine 2D-Videoanalyse durchgeführt um zu untersuchen, ob die benthische Lebensweise der harpacticiden Copepoden ein Problem für die pelagisch lebenden Fischlarven in Bezug auf Fangerfolg und Energieverbrauch darstellt (Kapitel 3). Benthische Copepoden wurden von den Fischlarven wahrgenommen. Aber die Fischlarven hatten seltener Futter im Darm, wenn sie mit Copepoden anstatt mit *B. plicatilis* gefüttert wurden. Dies verbesserte sich jedoch mit Entwicklung der Fischlarve. Die Verfütterung der Copepoden mittels eines schwimmenden Siebes verbesserte den Fangerfolg und verringerte wahrscheinlich den Energieverbrauch der Fischlarven.

Abschließend lässt sich sagen, dass harpacticide Copepoden ein hochwertiges Futter für marine Fischlarven sind, aber ihre Verdaubarkeit ist ein kritischer Punkt, der bei künftigen Evaluierungen neuer Arten und bei Fütterungsversuchen berücksichtigt werden sollte. Obwohl harpacticide Copepoden in relativ hohen Dichten kultiviert werden können, werden nicht so hohe Dichten erzielt wie mit *B. plicatilis*. Somit werden Copepoden eher eine Nahrungsergänzung als das alleinige Futter für Fischlarven darstellen.

Introduction

Introduction

Introduction

Aquaculture

To meet the increasing demand for fish caused by population and economic growth (Wijkstrom, 2003), the aquaculture production is increasing from being negligible in the 1980ies to 40.3% of the world fish supply in 2010. The capture based fishery is stagnating since the last decades due to fishery restrictions and declining natural fish stocks (FAO, 2004). The catching yield of some species is even decreasing due to overfishing. Consequently, aquaculture is nowadays the fastest growing sector in food industry (FAO, 2012) and is defined as "the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated" (FAO, 1997). In total, capture fishery and aquaculture provided 148.5 Mio tonnes of fish in 2010, resulting in a per capita supply of 18.6 kg (FAO, 2012).

Carp and Tilapia are the main farmed fresh water species and salmon and milkfish are the main marine species. The European aquaculture production is just a small contribution (4.2%) to the world total production of 59.9 Mio tonnes, with Norway being the main contributor, followed by Spain and France. Norway is the dominant producer of Atlantic salmon. The contribution of German aquaculture production is low with the focus on fresh water species, like rainbow trout and carp (Statistisches-Bundesamt, 2012). The European Union is the biggest importer of fish products owing to its increasing demand of fish. In general, fish provides high digestible protein and a high amount of polyunsaturated fatty acids, especially "omega-3"-fatty acids (Tocher, 2009) and they are important to fulfil the dietary demand of iodine (Fuge, 2007). To increase the European fish production and its competitiveness, the European fishery fund (EFF) was established in 2007.

Larviculture

The development of marine aquaculture resulted in an increasing demand of juveniles in marine hatcheries (Zambonino Infante and Cahu, 2001). Overall, the larval rearing is one of the most critical stages in fish production. The digestive tract of marine fish larvae is immature at hatching (Zambonino Infante and Cahu, 2001), the pigmentation of the eye is still under development at first feeding (Chesney, 2007) and

the prey perception distance increases with larval size (Miller et al., 1988). Therefore, the moment after yolk sac exhaustion, when the fish larva depends on exogenous food sources for energy and nutrients, is crucial for growth, development and survival.

In general, fish larvae are pelagic and are feeding in the natural environment on protozoa, copepods, mollusc larvae, appendicularians, rotifers, marine cladocerans and phytoplankton (Arthur, 1976; Hunter, 1980; Turner, 1984). The ingestible prey size of fish larvae is restricted by the size of their mouth gap. Thus, smaller fish larvae have a smaller prey spectrum than bigger ones. In nature, fish larvae encounter a diverse food and size spectrum, but they may also encounter a low prey density and are vulnerable to predation. To find adequate food at the right time without being predated is challenging. Consequently, just 1% of a brood stock reaches the juvenile stage (Houde, 2002).

Under aquaculture hatchery conditions, predation on larvae and low prey availability do not occur. However, the main disadvantage of hatcheries is the limited size spectrum offered to fish larvae, with the rotifers *Brachionus plicatilis* (299 ± 1.5 µm) and *Brachionus rotundiformis* (148.7 ± 1.3 µm) (Ciros-Pérez et al., 2001) and the nauplii of the brine shrimp *Artemia* sp. (428 – 515 µm, (Dhont and Van Stappen, 2003)) being the most common used prey types. The advantage of *Artemia* sp. is that no year-round production is necessary as the eggs can be stored and the nauplii can be produced on demand one day prior to feeding. The rotifer species, *B. plicatilis* and *B. rotundiformis* are relatively easy to rear and can be cultured in high densities. They are slow cruising pelagic organisms and can reproduce sexually and asexually (Lubzens et al., 2001).

In 2010, about 600 different fish species were cultured (FAO, 2012). They have diverse needs and food requirements, i.e. sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) larvae, two mediterranean species are reared at around 15°C and hatch with a larval length of 3 – 4 mm (Moretti et al., 1999), whereas halibut larvae (*Hippoglossus hippoglossus*) are reared at 4 – 7°C and hatch with a larval length of 6 mm (Kjørsvik et al., 2004). Larvae of *H. hippoglossus* are fed with *Artemia* sp., whereas the first two species need the smaller live feed *Brachionus* sp. at first feeding (Moretti et al., 1999; Kjørsvik et al., 2004). However, the rearing of these species with these live feeds can result in malformations, impaired vision and malpigmentation (Shields et al., 1999; Koumoundouros et al., 2002; Fernández et al., 2008). Several studies reported a higher nutritional value of copepods as food organisms for fish larvae compared to rotifers or *Artemia* (Evjemo et al., 2003; Hamre et al., 2008b; van der Meeren et al., 2008). However, not only nutrients decide about the suitability of a prey.

Prey suitability

The suitability of prey organisms depends on several factors, such as swimming behaviour of larvae and prey, prey digestibility and nutritional value.

Larval foraging behaviour and capture success

The larval predation process involves several phases: search, perception, attack and capture. The foraging behaviour of fish larvae have been described as saltatory (pause-travel) (Browman and O'Brien, 1992a; b; Galbraith et al., 2004) or as cruising (MacKenzie and Kiørboe, 1995; Mahjoub et al., 2012). Saltatory predators are searching while pausing, whereas cruising predators are searching while swimming (O'Brien et al., 1990). Fish larvae are mostly visual predators, but food detection also occurs by mechanical and chemical stimuli. Neuromasts are responsible to detect hydromechanical signals. Nitrogenous and amphoteric substances with low molecular weight, like amino acids are chemical signals, which stimulate olfactory ciliated receptors (Dempsey, 1978; Hara, 1993) and influence the swimming behaviour of fish larvae (Døving et al., 1994). While prey detection by olfaction can occur at larger distances, detection by sight occurs at relative low distances of around 0.5 to 1 body lengths (Miller et al., 1988). The increase of lens size and the development of the retina enhance the visual field and acuity with ontogeny (Yúfera, 2011). Herring larvae (*Clupea harengus*), which are used as a model organism in this thesis, are categorized as cruising predators (MacKenzie and Kiørboe, 1995). The visual field of herring larvae is in front and above, but prey items below them are not detected (Rosenthal and Hempel, 1970). Overall, the feeding success of *C. harengus* is low at first feeding (Rosenthal and Hempel, 1970; Blaxter and Staines, 1971) but increases with maturation of the sensory and locomotory system (Hunter, 1972; Chesney, 2007). The prey encounter rate increases with the swimming speed of prey and predator (Kiørboe and Visser, 1999). The predator covers a higher volume in shorter time and a faster prey produces higher hydromechanical signals which are perceived from a further distance by the predator. Furthermore, the perceiving distance increases with size and contrast of the prey (Buskey, 1994).

Once the prey item is perceived, the capture success of fish larvae is influenced by prey escape response (Buskey et al., 1993; Titelman and Kiørboe, 2003), prey swimming behaviour (Viitasalo et al., 1998) and prey visibility (Eggers, 1977). Fast moving copepods cause a better prey perception by the predator than slow moving rotifers, but copepods also show better escape responses (Beck and Turingan, 2007). The benthic behaviour of harpacticoids can lead to low prey availability for the pelagic fish larvae. An

improvement of the availability might be the copepod rearing method described by Kahan et al. (1982), who tested the rearing of harpacticoid copepods in a floating sieve directly in the fish rearing tank. The sieve had a mesh size through which naupliar stages can fall to be directly available for the fish larvae.

Digestion

The digestive process is decisive for the nutrient absorption of the successful captured prey. The prey is swallowed in one piece and is being digested in the intestine, since most fish larvae are altricial and lacking a stomach during their first weeks of life until metamorphosis (Rønnestad and Morais, 2008). The digestive tract is functionally differentiated into bucco-pharynx, oesophagus, stomach anlage, intestine and anus at first feeding. The absorptive capacity increases through elongation and mucosal folding of the intestine (Rønnestad et al., 2013). Liver, gall bladder and pancreas are accessory digestive organs. The liver is producing bile, which is utilized in the emulsification of lipids and the gall bladder storages and releases the bile in the gut. The endocrine pancreas releases metabolic hormones such as insulin into the plasma (Rønnestad and Morais, 2008). The digestion itself is performed by enzymes of the exocrine pancreas and the intestine. Pancreatic enzymes such as trypsin, lipase and amylase are released in the intestinal lumen, whereas cytosolic and brush border membrane enzymes are produced directly in intestinal cells. Pancreatic and cytosolic enzymes are mostly present at first feeding (Ribeiro et al., 1999; Zambonino Infante and Cahu, 2001). Concentrations of brush border enzymes are increasing with the folding of the mucosa and the development of microvilli at the luminal surface, meanwhile the activities of certain cytosolic enzymes are decreasing with larval development (Cahu and Zambonino Infante, 1995). The rise or decline of enzymes is genetically programmed, but the diet can modulate the plateau levels of enzymes and can include an earlier maturation of enterocytes, which are responsible for nutrient absorption (Zambonino Infante and Cahu, 1999; Buchet et al., 2000).

In the early stages of fish larvae, trypsin is the major pancreatic enzyme. Trypsin hydrolyses protein and plays a key role in activating other enzymes (Rønnestad et al., 2013). The stimulation is both under hormonal and neural control. In general, the secretion of enzymes is stimulated by the hormone cholecystokinin (CCK), which is released by chemical triggers in the intestine (Rønnestad et al., 2007). In humans, the presence of fats and proteins in the intestine is responsible for this stimulation (Chandra and Liddle, 2009). Consequently, the type and amount of food influences the trypsin

concentration (Pedersen et al., 1987; Pedersen and Andersen, 1992) and thus, tryptic activity can be a valuable indicator for the nutritional status of the fish larvae.

Nutritional aspect

Once a prey item is successfully digested, the extracted nutrients are important for the growth and development of the fish larva. Fish larvae are fast growing organisms with a high demand of amino and fatty acids. Free amino acids are used as metabolic fuel and are also needed for the larva's own protein synthesis. In general, fatty acids are constituents of different lipids. As part of triacylglycerols, they function mainly as a source of metabolic energy and are precursors of bioactive substances. As phospholipids, they form the lipid bilayer of all membranes. Biological processes, such as generation of adenosine triphosphate (ATP) and ion transport, are membrane associated and are regulated by the fluidity of the membrane, which in turn is determined by its fatty acid composition (Izquierdo and Koven, 2011; Wynn, 2011). Phospholipids are rich in polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (20:5ω3, EPA) and docosahexaenoic acid (22:6ω3, DHA). EPA and DHA originate in the marine food web mainly from microalgae. In microalgae EPA and DHA are synthesised by elongases and several desaturases (Δ6, Δ5 and Δ4) from α-linolenic acid (18:3ω3) (Fig. 1), whereas vertebrates are lacking the Δ4-desaturases (Bell and Tocher, 2009). Hence, vertebrates use the Sprecher-pathway by elongating 22:5ω3 to 24:5ω3, which is desaturated by a Δ6-desaturase to 24:6ω3 and finally transformed to 22:6ω3 (DHA) by β-oxidation (Sprecher, 2000). This way is quite complex due to a translocation from the cytosol into the peroxisome and back (Wynn, 2011), and is relatively limited in vertebrates. Therefore, both DHA and EPA are considered to be essential fatty acids for fish, since they cannot be synthesised in sufficient amounts from its precursor 18:3ω3 (Sargent et al., 1999b).
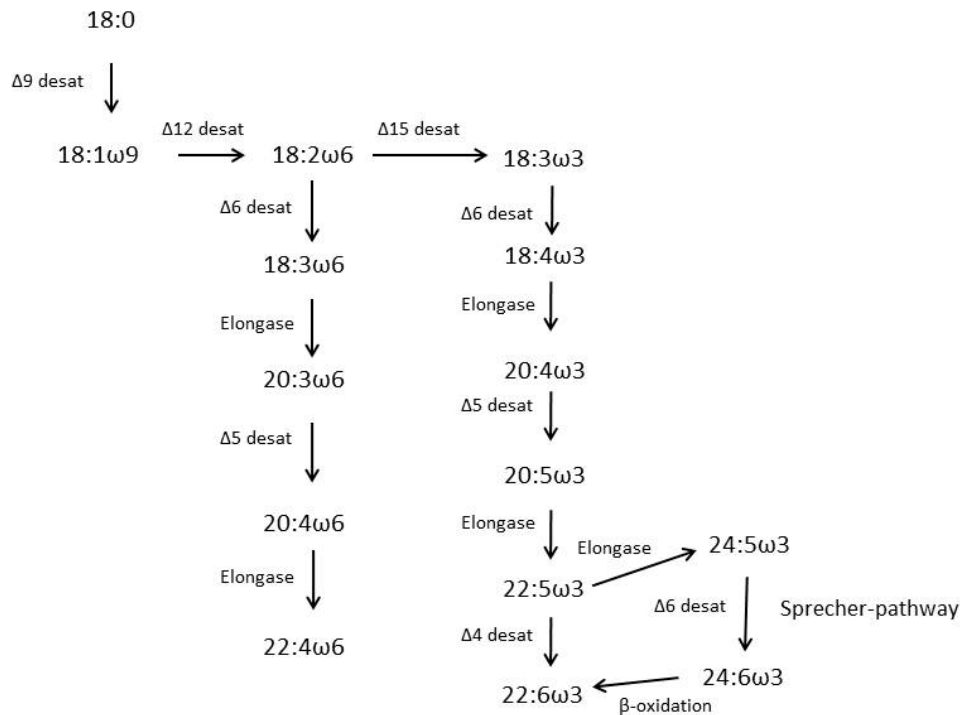
Introduction

Figure 1: Pathway of the ω3 and ω6-fatty acid biosynthesis (modified from Wynn (2011). desat =desaturase

In fish, high values of DHA are present in larval eyes (Benítez-Santana et al., 2007). A dietary deficiency in DHA can lead to impaired vision, as well as malpigmentation (Reitan et al., 1994) and skeletal deformities (Roo et al., 2009). EPA and arachidonic acid (ARA) are competitive in their role as precursors of eicosanoids. These are hormone-like substances which play a role i.e. in the stress vulnerability, the inflammatory and immune response of fish larvae. EPA derived eicosanoids are less bioactive than those produced by ARA (Tocher, 2003). Therefore, the ratio between these fatty acids is an important issue. Sargent et al. (1995) emphasised that the ratio of DHA:EPA:ARA is as important as the amount and suggested an optimal ratio of 10:5:1, based on different studies.

Copepods are rich in essential fatty acids and they have a high content of phospholipids (McKinnon et al., 2003). Phospholipids increase the efficiency of the dietary fatty acid transport from the intestine to the rest of the body (Tocher et al., 2008) and are therefore a superior source of essential fatty acids and energy (Coutteau et al., 1997). To optimise the nutritional value of rotifers and *Artemia*, both are routinely enriched with a lipid emulsion prior to be supplied in the larval fish tank (Fernández-Reiriz et al., 1993; Dhert et al., 2001). However, these enrichments are costly and often result in an increase in triacylglyceroles but not in phospholipids (Coutteau and

Sorgeloos, 1997; Rainuzzo et al., 1997). Furthermore, copepods probably offer an optimal protection of polyunsaturated fatty acids (PUFA) by natural antioxidants against peroxidation and they deliver optimal levels of natural antioxidants to the larvae (Sargent et al., 1997).

A diet solely based on copepods or as a food supplement to rotifers or *Artemia* resulted in an improved growth rate, higher survival and a higher rate of normal pigmentation of fish larvae (Shields et al., 1999; Payne et al., 2001; Schipp, 2006; Busch et al., 2010). Due to the evidence that copepods have superior food quality compared to rotifers and *Artemia*, it is fundamental to optimise the rearing of marine copepods in order to fulfil nutritional requirements of hatchery reared fish larvae.

Copepods

Copepods are the natural prey of many fish larvae (Hicks and Coull, 1983; Turner, 1984; 2004). They are also regarded as the insects of the sea in terms of size, diversity and abundance (Huys and Boxshall, 1991). Prior to reaching the adult stage, copepods run through 6 naupliar stages and 5 copepodite stages, providing a wide size range with only one species (Fig. 2). This makes copepods highly suitable as prey item for aquaculture hatchery purposes, as the cultivation of only one species results in the availability of a broad spectrum in prey size. The class Copepoda consists of 10 orders, of which Calanoida, Harpacticoida and Cyclopoida are the three orders commonly used in aquaculture.

Figure 2: Three developmental stages of *Tachidius discipes* (nauplius (156 ·116 µm, length·width), copepodites (306·146 µm), adult female (510·180 µm)).

Calanoids are pelagic and are mostly selective suspension or ambush feeders (Paffenhöfer et al., 1982; Kiørboe et al., 1996). Rearing fish larvae with calanoid copepods has positive effects on survival, pigmentation and retinal morphology (Shields et al., 1999). However, the main disadvantage of this order is the low rearing density,

since fecundity is reduced at high culture densities (Peck and Holste, 2006). In addition, calanoid and cyclopoid copepods are sensitive to handling procedures due to their fragile body. Harpacticoid copepods are mainly surface feeders (Hicks and Coull, 1983), can be cultured in higher densities than calanoid copepods (Støttrup, 2000), are tolerant to salinity and temperature changes and feed on diverse food sources like microalgae, ciliates, fungi and yeasts and bacteria as aggregates or as detritus associates (McIntyre, 1969; Hicks and Coull, 1983). Even a successful cultivation with vegetables and rice grains was reported (Betouhim-El and Kahan, 1972; Kahan, 1979).

Furthermore, harpacticoid copepods are considered to have the ability to synthesise EPA and DHA along the biosynthesis-pathway (Fig. 1). So far, it is unknown whether the direct way or the Sprecher-pathway is used by the invertebrates including harpacticoid copepods (Bell and Tocher, 2009). However, this would have the advantage that these copepods can upgrade low nutritional algae, such as *Dunaliella tertiolecta,* which contain neither DHA nor EPA, but are easy to culture.

Therefore, the focus was set on harpacticoid copepods in this study. The adult size of harpacticoids ranges from 0.2 to 2.5 mm and free-living individuals occupy diverse habitats, including interstitial, phytal and epibenthic living modes (Hicks and Coull, 1983). To date the number of tested copepods in aquaculture is relatively small and the main investigated species are: *Amphiascoides atopus*, *Amonardia* sp., *Euterpina acutifrons* (pelagic), *Tigriopus* spp., *Tisbe furcata* and *Tisbe holothuriae* (Fleeger, 2005). As production and nutritional value differs among species, a copepod screening is required. Uhlig (1984) emphasised several criteria for species used for aquaculture purposes:

- High tolerance to changes in salinity and temperature
- Short life cycle
- High reproductive capacity
- Tolerance of high culture densities
- Acceptance of diverse food sources

Aim of the study

The aim of this thesis is to improve the rearing of harpacticoid copepods for marine fish larvae. This includes the growth of the copepod population itself, as well as the suitability for fish larvae.

The first chapter addresses the growth performance and food quality of two different harpacticoid species. After screening several species from the Kiel fjord, *Tachidius discipes* is considered as a potential new candidate for aquaculture purposes and is compared with *Tisbe* sp., a species already tested in larval feeding experiments. Besides labour, food is generally the largest cost factor in the aquaculture business, making up between 30 and 60% (Southgate, 2003). Therefore, the development index and reproduction in response to five different microalgae is analysed and the optimal algal food concentration determined. Furthermore the transfer of fatty acids from alga to copepod is analysed by providing microalgae varying in cell size and fatty acid composition. The suitability of these copepods in terms of fatty acid composition especially EPA and DHA is discussed.

In chapter 2, a larval feeding experiment is conducted to test the suitability of *T. discipes* as food for fish larvae. *Clupea harengus* is used as a model organism. To study the effect of different prey items on the development of fish larvae and larval enzyme activity, copepods, rotifers and a mixture of both are offered to larvae of *C. harengus*. The mixture is provided to investigate whether a supplement of copepods is sufficient to result in improved larval condition. Based on the low growth performance of copepod-fed larvae, the digestibility of prey items typically used in aquaculture is also explored.

In addition, the foraging behaviour of herring larvae in response to harpacticoid copepods and rotifers is investigated using video observations (Chapter 3). An indirect copepod supply method is tested, where copepods are provided via a floating sieve. This method is assumed to improve the availability of harpacticoid copepods.

Chapter 1

Chapter 1

Effect of algal species and concentration on development and fatty acid composition of two harpacticoid copepods, *Tisbe* sp. and *Tachidius discipes*, and a discussion about their suitability for marine fish larvae

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Effect of algal species and concentration on development and fatty acid composition of two harpacticoid copepods, *Tisbe* sp. and *Tachidius discipes*, and a discussion about their suitability for marine fish larvae

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Abstract

We compared the development and fatty acid content of the harpacticoid copepods *Tachidius discipes* and *Tisbe* sp. fed with different microalgal species (*Dunaliella tertiolecta*, *Rhodomonas* sp., *Phaeodactylum tricornutum*, *Isochrysis galbana*, and a concentrate of *Pavlova* sp.), which differed in cell size and fatty acid composition.

Tisbe could develop in 11 days with every alga to the same average stage, whereas *Tachidius* developed poorly when fed with *Isochrysis* and *Dunaliella*. Feeding with *Phaeodactylum* resulted in a fast development of both copepods at low algal concentrations. However, reproduction was higher with *Rhodomonas* as food than with the other algae.

Fatty acid compositions of copepods were influenced by their food source, but both were able to convert DHA and EPA from precursors. *Tachidius* fed with *Rhodomonas* or *Phaeodactylum* was closest to the DHA:EPA:ARA-ratio of 10:5:1 considered optimal for some marine fish larvae. *Tachidius* showed similar development and reproduction capacity as *Tisbe,* but requested higher absolute fatty acid contents in the diet. *Tisbe* was superior in the utilisation of bacteria as additional food source and the bioconversion of precursor fatty acids. *Phaeodactylum* and *Rhodomonas* are recommendable food sources for both copepod species, but *Phaeodactylum* is more easily cultured.

1.1. Introduction

Marine fish larviculture relies on easily reared live food such as rotifers and *Artemia* and, since these are deficient in highly unsaturated fatty acids, they are routinely enriched prior to being fed to fish larvae (Fernández-Reiriz et al., 1993; Dhert et al., 2001). These enrichments often result in an increase in triacylglyceroles but not in phospholipids (Coutteau and Sorgeloos, 1997; Rainuzzo et al., 1997).

Copepods are the natural food of many fish larvae (Sibert et al., 1977; Alheit and Scheibel, 1982; Turner, 1984; 2004) and they provide a high nutritional value (Shields et al., 1999; Evjemo et al., 2003). Moreover, in copepods phospholipids predominate compared to triacylglycerols (Sargent et al., 1997). Phospholipids increase the efficiency of transport of dietary fatty acids from the intestine to the rest of the body (Tocher et al., 2008) and are therefore a superior source of essential fatty acids and energy (Coutteau et al., 1997).

Fatty acids are one of the components which contribute to successful growth of heterotrophic organisms, as they are required for normal cell membrane functions (Sargent et al., 1995). Fatty acids like eicosapentaenoic acid (20:5ω3; EPA), docosahexaenoic acid (22:6ω3; DHA) and arachidonic acid (20:4ω6, ARA) are essential fatty acids (EFA) for most metazoan consumers and have to be incorporated with food. The ratio between these EFAs and the amount of each play an important role in the successful rearing of marine fish larvae (Sargent et al., 1995).

Some fish species have poor larval survival using the traditional live feed of rotifers and *Artemia*. For instance, grouper larvae have a quite small mouth gap and have a higher feeding success with copepod nauplii compared to rotifers (Doi et al., 1997). Red snapper was only ingesting copepods, even when copepods and rotifers were present (Zavala-Leal et al., 2012).

Usually the demand for copepods in larviculture is covered by net-catches of natural zooplankton, which are most often dominated by calanoid copepods. However, it is difficult to control the quality of natural zooplankton.

To rear pelagic copepods directly under controlled conditions at the hatchery site, high water volumes are required, since reproduction of calanoid copepods is reduced at high densities (Peck and Holste, 2006). Benthic harpacticoids can be reared in higher densities than calanoids (Støttrup, 2000), are tolerant to salinity and temperature changes and they are able to feed on diverse food sources including microalgae, bacteria, organic matter, ciliates and detritus (McIntyre, 1969).

Hence, we focussed on harpacticoid copepods as food source, *Tisbe* sp. and *Tachidius discipes*. While *Tisbe* spp. is a popular species in feeding experiments (Heath and Moore, 1997; Støttrup and Norsker, 1997; Olivotto et al., 2008a; Olivotto et al., 2008b), the suitability of *Tachidius discipes* for aquaculture purposes has not been explored yet to the best of our knowledge.

Besides labour, food is generally the largest cost factor in the aquaculture business, making up between 30 and 60% of the total cost (Southgate, 2003). Food production in a hatchery starts with algae for live feed. Algae are quite expensive to produce and often require a special nutrient medium containing trace metals and vitamins. Hence, it is crucial to know the optimal algal food concentration and to choose an algal species which results in good development and reproduction.

In the present study, we tested five algal species, differing in cell size, cell concentration and fatty acid composition, in respect to their effects on development, reproduction and fatty acid composition of two harpacticoid copepod species. We investigated the transfer and incorporation of algal fatty acids into harpacticoid copepods and as a result of this study, we propose a food chain which provides a suitable nutrition for fish larvae in aquaculture.

1.2. Material and Methods

1.2.1 Algal culture

Five different algae (*Rhodomonas* sp., *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Dunaliella tertiolecta* and a concentrate of *Pavlova* sp.) from different algal classes and with different fatty acid compositions (Table 1.1) were selected as food for the copepods. The culture medium, for all algal species except for *Pavlova* sp., was prepared by enriching filtered (0.2 μm) and autoclaved Baltic Sea water (18 ± 1 g L^{-1}) with trace metals and vitamins based on the modified Provasoli's enriched seawater medium (Provasoli, 1968; Ismar et al., 2008). Nitrogen and phosphorus were added as NaNO$_3$ and KH$_2$PO$_4$ at a concentration of 880 μmol L^{-1} and 36 μmol L^{-1}, respectively. The algae were cultured at 18°C, a light intensity of 100 μmol m^{-2} s^{-1} and at a light-dark cycle of 16h:8h. *Pavlova* sp. was offered in all experiments as a concentrate produced by BlueBioTech GmbH, Büsum, Germany, which aims at developing it for commercial use. Thus, differences to Pavlova as live algae may occur. Differences can be caused for example by leakage of nutrients during centrifugation or by the sudden salinity drop from 30 g L^{-1} in the concentrate to 18 g L^{-1} in the experiments. However, microscopical

observation did not reveal destroyed cells, but the unusual small cell size (Table 1.1) can be a first hint.

Table 1.1: Characteristics of the algal species used in this study

Algal species	Origin	Mean ± SD carbon content (pg C cell[-1]) N = 3	Mean ± SD cell diameter (µm) N = 6	Fatty acid contents (%)
Rhodomonas sp. (Chryptophyceae)	Isolated from Kiel fjord	67 ± 6	10.5 ± 2.0	8.7 EPA[4] 4.6 DHA[4]
Phaeodactylum tricornutum (Bacillariophyceae)	SAG1090-1b	11 ± 2	27.4 ± 2.8 (length) 2.7 ± 0.3 (width)	0 – 2.3 ARA[1,2] 13.6 – 30.5 EPA[1,2] 0.2 – 1.7 DHA[1,2]
Isochrysis galbana (Prymnesiophyceae)	Isolated from North Sea	8 ± 1	4.8 ± 0.8	15.4 – 20.4 DHA[1,2] 1.0 – 1.4 EPA[1,2]
Dunaliella tertiolecta (Chlorophyceae)	SAG13.86	113 ± 5	10.6 ± 2.1	no DHA and EPA[1]
Pavlova sp. (Pavlovophyceae)	BlueBioTech GmbH, Germany	5 ± 1	3.2 ± 0.6	0.3 – 3.7 ARA[1,2,3] 17.3 – 23.2 EPA[1,2,3] 9.3 – 17.0 DHA[1,2,3]

[1] Lang et al. (2011), [2] Patil et al. (2007), [3] Zhukova and Aizdaicher (1995), [4] Renaud et al. (1999)

1.2.2 Copepod culture

The present experiments used *Tachidius discipes*, a harpacticoid copepod isolated at the Kiel Bay, Germany at a sandy shore in June 2009 and *Tisbe* sp. isolated from a cultivation tank of *Acartia tonsa* in November 2009. Both have been kept in culture in our laboratory (GEOMAR, Kiel, Germany) since that time. *Tachidius* is an eulittoral species (Noodt, 1957) with a length of 0.5 mm and a generation time of 19.6 days at 20°C (Heip and Smol, 1976). *Tisbe* sp. is an eurytopic substratum generalist (Hicks, 1982) with a length of 0.7 mm and a generation time of 16.4 days at 20°C (Bergmans, 1981). The populations were maintained in crystallising dishes of 400 mL volume at 18 ± 1°C with a 12:12 h-L:D-period and a light intensity of 31 µmol m^{-2} s^{-1}.

We used a code for the different copepod – alga combinations throughout the manuscript, with following abbreviations: *Tisbe* (Ti), *Tachidius* (Ta), *Phaeodactylum* (Phaeo), *Rhodomonas* (Rhodo), *Dunaliella* (Duna), *Isochrysis* (Iso) and *Pavlova* (Pav). The generic names of these species or the abbreviations are used henceforth.

1.2.3 Experimental setup

<u>Growth and development</u>

The four different algal species, (*Phaeodactylum*, *Rhodomonas*, *Dunaliella*, *Isochrysis*) were kept in exponential growth phase by dilution every second day while the *Pavlova*-concentrate was used directly. Algae were offered in 6 different carbon concentrations (0.16, 0.24, 0.32, 0.64, 1.28, 2.56 mg C L^{-1}) to the nauplii of *Tachidius* and *Tisbe* starting with stage I or II. Higher concentrations (5.12, 10.24, 20.48 mg C L^{-1}) were offered when fed with *Pavlova*, due to low survival of *Tisbe* at lower concentrations. Seven days before the experiment started, the copepods were acclimatised to the corresponding algal species and were kept under food saturating conditions. Eventually, groups of 10 nauplii in triplicate for each algal species and concentration were cultivated in 10 mL filtered (0.2 µm) Baltic Sea water (17 ± 1 g L^{-1}) in 6-well culture plates, resulting in a density of 1 nauplius mL^{-1} or 1.04 nauplii cm^{-2}. The experiment was conducted at 18°C at a 12:12 h-L:D light regime and a light intensity of 31 µmol m^{-2} s^{-1}. Water exchange and restocking to initial algal concentration was done every second day. After eleven days, the development stage was determined, a stage value was assigned and the development index (DI) was calculated according to

$$(1) \qquad DI = \sum SV \cdot N^{-1},$$

where *SV* = assigned stage value (Table 1.2) and *N* = number of copepods.

Table 1.2: Assigned stage values (*SV*) for the different development stages of copepods.

Stage	Stage value	Stage	Stage value
N1	0	C1	5
N2	0	C2	6
N3	1	C3	7
N4	2	C4	8
N5	3	C5	9
N6	4	C6	10

N = naupliar stage, C = copepodite stage

To analyse the impact of algal food concentration on the development index, a nonlinear regression model was fitted as used in Knuckey et al. (2005):

$$(2) \qquad DI = DI_{max} + A \cdot \left(B^x\right),$$

where *x* = food concentration (mg carbon L^{-1}) and *A* and *B* = regression parameters.

The saturating food level was calculated by solving Equation 2 for *DI* = 99% of *DI$_{max}$*. In addition, the survival of copepods was determined at the end of the experiment.

Chapter 1

Reproduction

In this experiment, the effect of four microalgal species (*Rhodomonas*, *Phaeodactylum*, *Isochrysis* and *Pavlova*-concentrate) as food source on the nauplii production of the two copepod species were determined under food saturating conditions (4, 4, 4, 7, 14 and 18 mg C L^{-1} (*Rhodomonas*, *Phaeodactylum*, Ti-Iso, Ta-Iso, Ta-Pav and Ti-Pav, respectively)) in a 7-day experiment at 18°C at a 12:12 h-L:D light regime and a light intensity of 31 µmol m^{-2} s^{-1}. The reproduction with *Dunaliella* could not be analysed due to low number of egg-bearing females. After acclimatising the copepods to the different algae for ten days, three females, carrying their first egg-sacs, were transferred to one well (10 mL) of a well plate (N$_{wells}$ = 18, in copepod cultures with *Pavlova* the number of egg-carrying females was low, consequently the number of replicates was lower: N$_{wells}$ = 9 (Ta-Pav), N$_{wells}$ = 6 (Ti-Pav)) filled with 300 mL filtered (0.2 µm) Baltic Sea water (17 ± 1 g L^{-1}), which resulted in a density of 0.3 females mL^{-1} or 0.3 females cm^{-2}. Each day the hatched nauplii were counted and removed. Every second day the females were transferred to new plates with the initial algal concentration.

Fatty acid conversion

The effect of food source on the fatty acid composition of the two copepod species *Tisbe* and *Tachidius* was determined in a 30-day experiment at 18°C at a 12:12 h-L:D light regime and a light intensity of 31 µmol m^{-2} s^{-1}. It was started with around 50 egg-bearing females in each glass dish (ø 14 cm) filled with 300 mL filtered (0.2 µm) natural Baltic Sea water (17 ± 1 g L^{-1}). Five different algae (*Rhodomonas*, *Phaeodactylum*, *Isochrysis*, *Dunaliella, Pavlova*) were offered at food saturating conditions. Water exchange was conducted every tenth day. The five treatments were run in triplicates. The samples were filtered onto precombusted GF/F-filters (Whatman, 25 mm diameter) and stored at -80°C until further analysis. Because the two copepod species were tested successively, with the exception of *Pavlova* as the diet, the fatty acid content of the algae changed between the two experimental runs. Therefore to compare the fatty acid pattern between the two copepods species, the differences (*DF*) between copepod and algal species were calculated as

(3) $DF = FA_{Copepod} - FA_{Alga}$,

where *DF* = difference, $FA_{Copepod}$ = fatty acid content of the copepod (ng µg biomass-C^{-1}) and FA_{Alga} = fatty acid content of the alga (ng µg biomass-C^{-1}).

1.2.4 Analytical procedure

To analyse the carbon content of algae and copepods, algal cultures and copepods were filtered onto precombusted GF/F-filters (Whatman, 25 mm diameter), dried overnight and then analysed with an organic elemental analyser (FLASH 2000, Thermo).

Fatty acids were extracted overnight with a solvent mixture of chloroform:dichloromethane:methanol with a ratio 1:1:1. C13:0, C15:0, C17:0, C19:0 and C21:0 fatty acid methyl esters were added as internal standards. C23:0-fatty acid was added for esterification control. After separation into an organic layer and an aqueous layer by adding a 1-molar potassium chloride solution, sodium sulphate was added to the organic layer. After transferring the organic layer in a new glass cocoon, the fatty acids were converted to methyl esters at 50°C with a mixture of toluene and methanol which was supplemented with 1% concentrated sulphuric acid. After creating two layers with addition of 5% sodium chloride solution and hexane, the hexane phase was transferred to a new glass cocoon and evaporated under reduced pressure until dryness. The extract was redissolved with hexane to a final volume of 100 μL (modified after Christie, 1989).

The fatty acid methyl esters were analysed in a gas chromatograph (Trace GC-Ultra, Thermo Fisher Scientific) equipped with a flame ionization detector and a TR-FAME-column (10 m, 0.1 mm i.d., 0.20 μm film) with hydrogen as the carrier gas. The temperature programme started at 50°C for 1 min, increased by 30°C min^{-1} to 150°C, then 4°C min^{-1} to 180°C and 30°C min^{-1} to 240°C. Peaks were integrated using Chromcard software (Thermo Fisher Scientific) and identified with reference to known standards. The focus was set on the ω3 and ω6 fatty acids, but all fatty acids were included for the calculation of the total fatty acid content. Fatty acid values were biomass-normalized (ng FA μg C^{-1}).

1.2.5 Statistical analyses

Prior to statistical analyses data were tested for normality and homogeneity of variances. If these criteria were not fulfilled a transformation was performed.

Effects of the diet and copepod species on the DI and nauplii production were tested by a two way ANOVA. Differences in survival were tested by a three way ANOVA, including the food concentration as a factor. A post-hoc test (Tukey unequal N HSD was conducted to analyse the differences in nauplii production in detail using STATISTICA 8. Level of significance was set at $p < 0.05$.

Since not all fatty acids and fatty acid groups fulfilled the requirement of homogeneity of variances, a PERMANOVA was conducted to analyse single fatty acids and ratios statistically. Similarity matrices were computed using Euclidean distance. For the pair-wise test the Monte-Carlo p-values were used due to small numbers of permutation (Anderson et al., 2008). Differences between diet and copepod were tested by using the original fatty acid values per carbon biomass. The significance level was set at $p < 0.05$. To investigate the overall fatty acid variation between algae and copepod and among copepod species, multivariate analyses were conducted. Fatty acids which were present in more than five treatments were square-root transformed to even out rare and dominant fatty acids. A Bray-Curtis similarity matrix was calculated and analysed by multidimensional scaling (MDS) and cluster analyses. Similarity percentages routine (SIMPER) was conducted to determine the dissimilarities between treatments and the fatty acids which contribute the most to those differences. Primer 6.0 (Primer-E Ltd) was used for all statistical tests with fatty acids.

Unless otherwise stated, all values are presented as mean value ± standard deviation.

1.3. Results

1.3.1 Growth and development

The development index (DI) of both copepod species increased with increasing food concentration with a tendency to level off at high food concentrations (Fig. 1.1). Fitting Equation 2 to the results yielded the parameter estimates shown in Table 1.3.
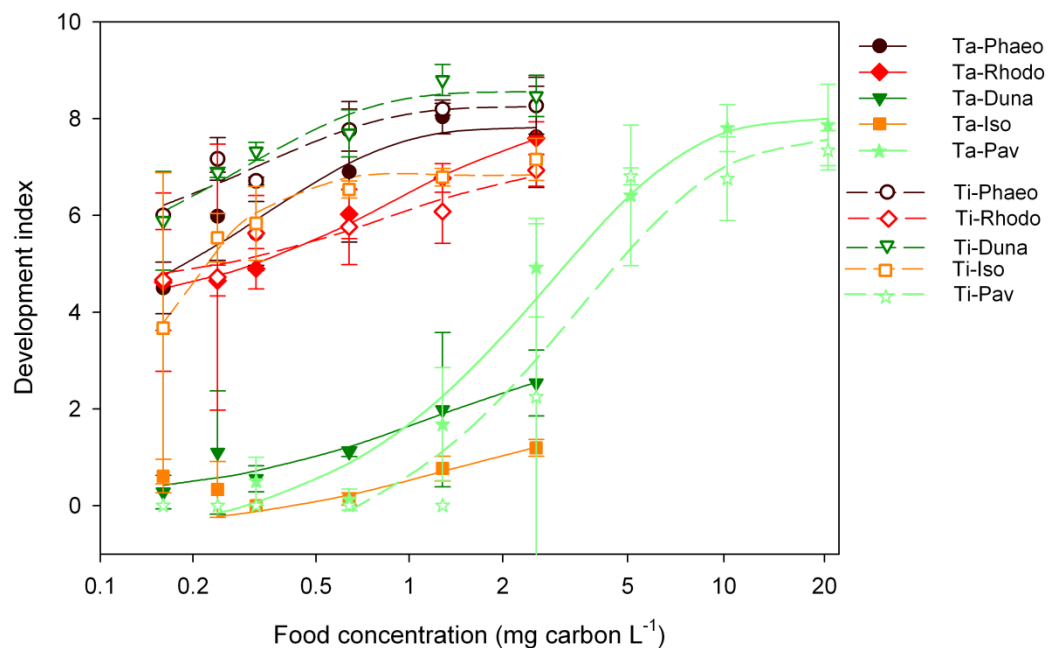

Figure 1.1: Development index of *Tachidius discipes* (Ta) and *Tisbe* sp. (Ti) as a function of algal food concentration (mg C L^{-1} in Weibull-log-scale) for the five different algae used after eleven days of feeding on the respective food species. Symbols display the average of three measured replicates and standard deviation. Lines display the fitted regression with parameters shown in Table 3. Rhodo = *Rhodomonas* sp., Phaeo = *Phaeodactylum tricornutum*, Pav = *Pavlova* sp., Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana.*

Table 1.3: Parameters ± standard error of the regression model $DI = DI_{max} + A \cdot \left(B^{x} \right)$, R^2 of the whole model and the calculated concentration of saturation, N = 3.

Copepod - Alga	Maximum development index (DI_{max})	A	B	R^2 of the model	Calculated concentration of saturation (mg C L^{-1})
Tachidius - Phaeo	7.82 ± 0.41	-4.94 ± 1.49	0.05 ± 0.08	0.68	1.39
Tachidius - Rhodo	7.85 ± 0.81	-3.98 ± 0.68	0.35 ± 0.22	0.74	3.72
Tachidius - Duna	2.93 ± 1.27	-2.84 ± 1.02	0.45 ± 0.39	0.54	5.76
Tachidius - Iso	1.54 ± 0.43	-2.09 ± 0.30	0.49 ± 0.19	0.89	6.79
Tachidius - Pav	8.01 ± 0.37	-8.85 ± 0.43	0.71 ± 0.04	0.95	13.95
Tisbe - Phaeo	8.25 ± 0.19	-3.36 ± 0.75	0.04 ± 0.05	0.80	1.19
Tisbe - Rhodo	6.98 ± 1.22	-2.59 ± 1.05	0.34 ± 0.53	0.31	3.31
Tisbe - Duna	8.56 ± 0.23	-4.33 ± 1.10	0.03 ± 0.04	0.79	1.13
Tisbe - Iso	6.84 ± 0.43	-11.96 ± 10.79	0.0002 ± 0.001	0.49	0.61
Tisbe - Pav	7.61 ± 0.61	-9.17 ± 0.75	0.76 ± 0.05	0.89	17.69

Phaeo = *Phaeodactylum tricornutum*, Rhodo = *Rhodomonas* sp., Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana*, Pav = *Pavlova* sp.

The five algal species (ANOVA: F = 19.58, p < 0.001) and the copepod species (ANOVA: F = 62.69, p < 0.001) had significant effects on the development index of the two copepod species *Tachidius* and *Tisbe*. The treatments in this study can be divided into three different groups (Fig. 1.2). *Tisbe* (Ti-Rhodo, Ti-Phaeo, Ti-Duna, Ti-Iso) and *Tachidius* (Ta-Rhodo and Ta-Phaeo) could develop in 11 days to a DI_{max} of around 7.5 which correspond to stage C3 – C4 with comparably low algal concentrations (0.61 – 3.72 mg C L^{-1}) (Table 1.3). *Tachidius* fed with *Dunaliella* or *Isochrysis* required higher algal concentrations (5.76 and 6.79 mg C L^{-1}) to reach the DI_{max} (2.93 and 1.54, respectively). These DI_{max} were significantly lower than the others mentioned before. The third group comprised *Tisbe* and *Tachidius* fed with a *Pavlova*-concentrate. With *Pavlova* the smallest alga tested, they could develop in the experimental period as far as the first group, but they needed a higher algal concentration (17.69 and 13.95 mg C L^{-1}, resp.) to be food saturated.
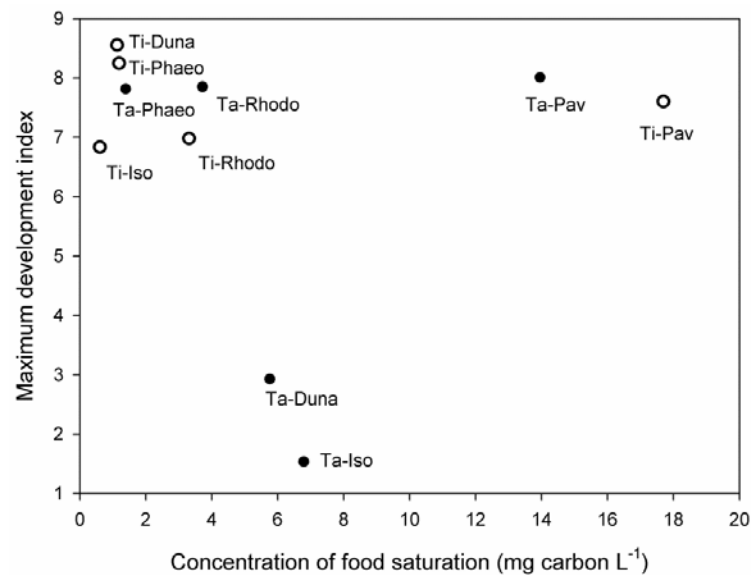
Figure 1.2: Maximum development index of *Tachidius discipes* (Ta) and *Tisbe* sp. (Ti) versus algal concentration of food saturation (mg C L^{-1}) calculated with the nonlinear regression model (see Equation 2). Rhodo = *Rhodomonas* sp., Phaeo = *Phaeodactylum tricornutum*, Pav = *Pavlova* sp., Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana.*

The copepod survival generally increased with increasing algal concentration (Fig. 1.3), though *Tachidius* showed a high survival rate also at low concentrations when fed with *Phaeodactylum* or *Rhodomonas*, indicating an interaction between diet, food concentration and copepod (ANOVA: F = 5.44, p < 0.0001,). *Tachidius* fed with *Isochrysis* at the highest concentration just obtained a survival of 53.3 ± 11.6% after 11 days, all other treatments resulted in a survival between 80.0 ± 10.0% and 96.7 ± 5.8%. *Tisbe* fed with *Pavlova* could only survive with algal concentrations of 2.56 mg C L^{-1} and higher.
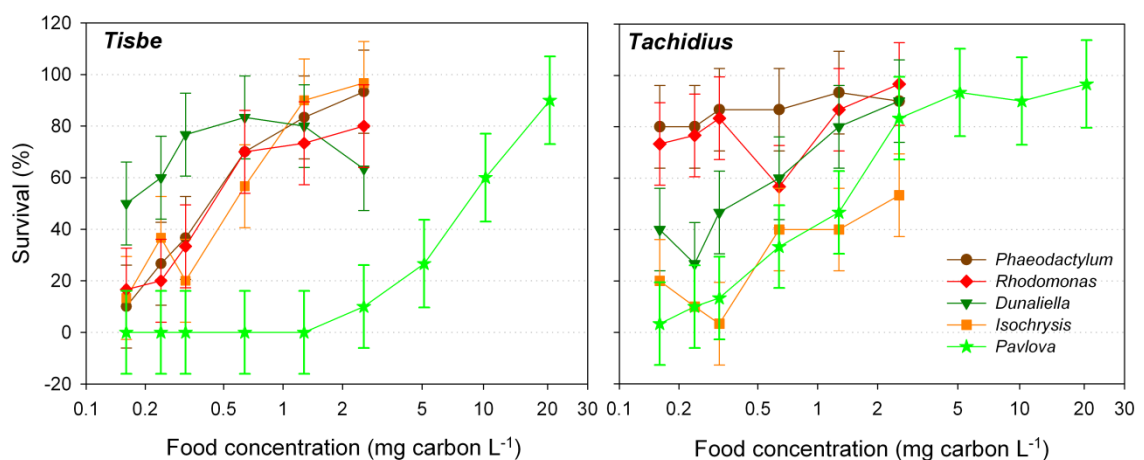

Figure 1.3: Survival (%) of *Tisbe* sp. and *Tachidius discipes* after 11 days as a function of algal concentration (mg C L^{-1} in Weibull-log-scale). Values are given as least square means ± 95% confidence interval (N = 3).

1.3.2 Reproduction

Algal species had a significant effect (ANOVA: F = 17.54, p < 0.001) on daily naupliar production per female (Fig. 1.4). No significant differences between the two copepod species could be observed (ANOVA: F = 0.68, p = 0.41), when feeding on the same algal species, except for *Isochrysis*. Reproduction of *Tachidius* fed with *Isochrysis* could not be tested because of the slow development of *Tachidius* feeding on *Isochrysis*. With *Dunaliella* both copepod species had extremely low reproduction, which could not be measured. *Tachidius* and *Tisbe* had the highest production of nauplii per female when feeding on *Rhodomonas* (9.73 ± 2.18 and 9.57 ± 1.93 nauplii female^{-1} day^{-1}, resp.). However, the difference in reproduction between *Rhodomonas* and *Phaeodactylum* was only significant with *Tisbe* (p < 0.05) but not with *Tachidius* (p = 0.25). The production of nauplii was not significantly different between the treatments when fed with *Phaeodactylum*, *Pavlova* or *Isochrysis*.
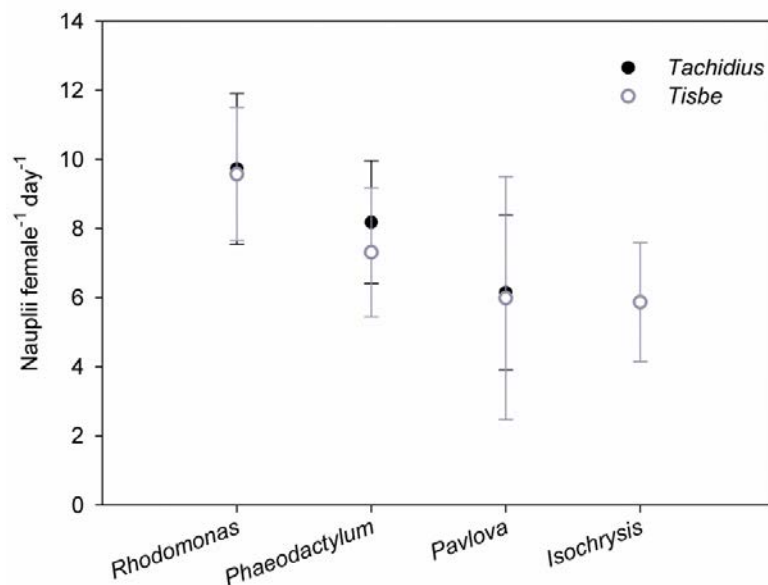

Figure 1.4: Daily naupliar production per female with standard deviation (N = 18, *Tachidius-Pavlova*: N = 9, *Tisbe-Pavlova*: N = 6) when feeding on the different algal species at food saturation levels.

1.3.3 Fatty acids

<u>Algae</u>

The figures 1.5 and 1.6 outline the composition of the ω6 and ω3 fatty acids. Since the feeding experiment with *Tisbe* was not conducted simultaneously to the one with *Tachidius,* except for *Pavlova* as the diet, the fatty acid composition of the same algal

species differed in some cases. However, the main pattern remained similar. *Phaeodactylum* contained the lowest level of total ω6. This group consisted mainly of 18:2ω6, whereas 18:3ω6 was most abundant in *Dunaliella* and 20:4ω6 (ARA) could be observed in high amounts in *Pavlova* (11.61 ± 0.82 ng µg C^{-1}) but only in traces in the other diets (0 - 0.76 ± 0.14 ng µg C^{-1}).

The total amount of ω3-fatty acids was higher than the level of the ω6-groups. 18:3ω3 was absent in *Phaeodactylum* but present in all other algae. *Dunaliella* did not contain any fatty acids with a longer chain length than 18 C-atoms. The ω3-group in *Dunaliella* comprised 16:4ω3, 18:3ω3 and 18:4ω3. Significant differences in the EPA content were found between all algal species, decreasing in the order: *Phaeodactylum*, *Pavlova*, *Rhodomonas*, *Isochrysis* and *Dunaliella*. *Isochrysis* had the highest amount of DHA, followed by *Rhodomonas*. *Phaeodactylum* and *Pavlova* contained significantly lower amounts of DHA than *Rhodomonas*, but significantly higher ($p < 0.005$) than *Dunaliella*. The DHA:EPA ratio differed between the algal species over a wide range from 0.03 ± 0.01 (*Pavlova*) to 26.33 ± 2.00 (*Isochrysis*).
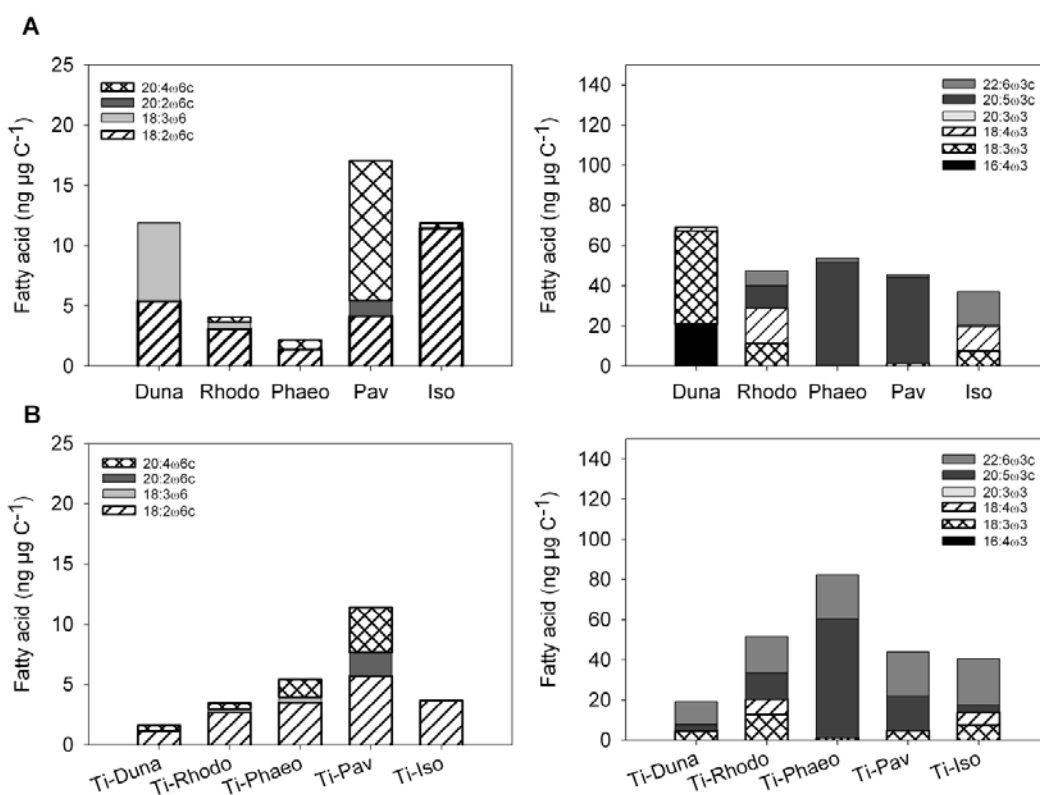
Fatty acid transfer

Figure 1.5: Amount of fatty acids of the ω6 (left side) and ω3-group (right side) (ng FA µg C^{-1}) in (A) five different algal species and in (B) *Tisbe* sp. (Ti) which fed on different algal species, N = 3, Ti-Phaeo: N = 2, (Rhodo = *Rhodomonas* sp., Phaeo = *Phaeodactylum tricornutum*, Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana*, Pav = *Pavlova* sp.)
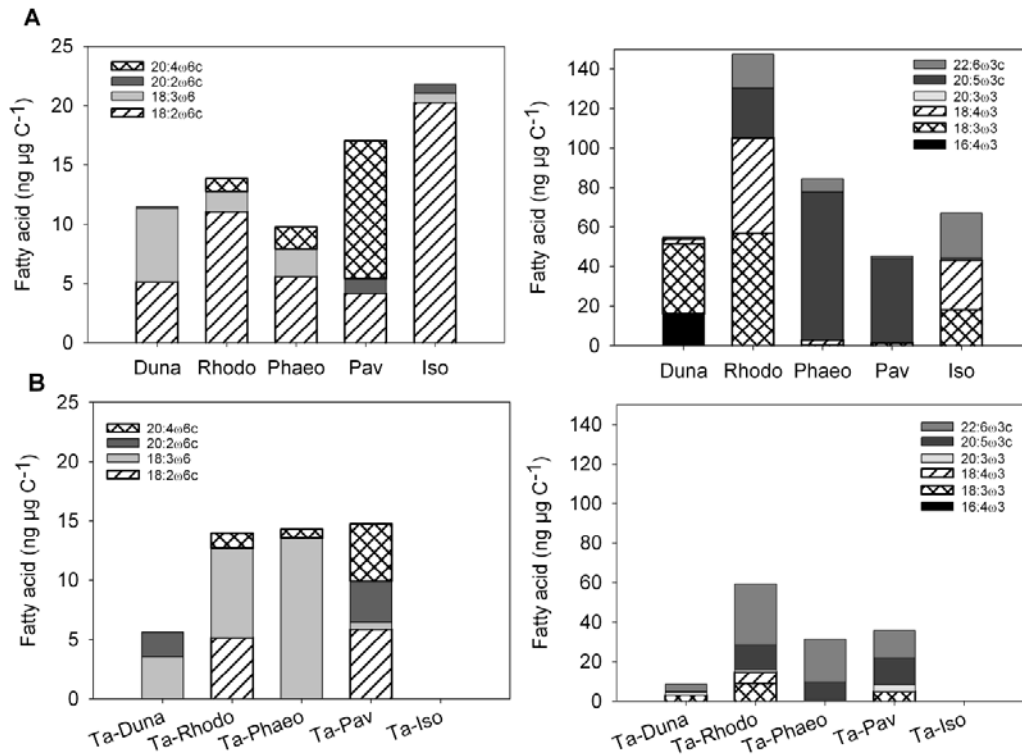
Figure 1.6: Amount of fatty acids of the ω6 (left side) and ω3-group (right side) (ng FA µg C^{-1}) in (A) five different algal species and in (B) *Tachidius discipes* (Ta), which fed on different algal species, N = 3, (Rhodo = *Rhodomonas* sp., Phaeo = *Phaeodactylum tricornutum*, Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana*, Pav = *Pavlova* sp.)

When copepods were feeding on *Dunaliella*, a chlorophyte without EPA and DHA, all polyunsaturated C18-fatty acids decreased in their carbon-specific amounts in the copepods relative to the algae (Fig. 1.5 and 1.6). At the same time, ARA, EPA and DHA increased significantly in *Tisbe* (Ti-Duna) (Table 1.4 and 1.5). In *Tachidius* (Ta-Duna) EPA and DHA also increased, however this increase was statistically not significant due to the large variability among replicates.

When fed with *Phaeodactylum*, 18:3ω3, ARA and DHA in *Tisbe* (Ti-Phaeo) increased significantly compared to the diet. In contrast, *Tachidius* (Ta-Phaeo) contained significantly less ARA and more 18:3ω6 compared to the diet. Ta-Phaeo also had a significantly higher content of DHA and a lower content in EPA than the diet.

When fed with *Isochrysis, Tisbe* (Ti-Iso) had a significantly lower content of 18:2ω6, ARA and 18:4ω3 and higher content of EPA than the diet, whereas the fatty acid content of *Tachidius* (Ta-Iso) could not be analysed due to low growth and reproduction.

When copepods were feeding on *Rhodomonas*, 18:3ω3 and 18:4ω3 decreased in *Tisbe* (Ti-Rhodo), while all other fatty acids of the ω3- and ω6-group did not change significantly. *Tachidius* (Ta-Rhodo) contained significantly less 18:3ω3, 18:4ω3 and EPA and more of 18:3ω6.

When feeding on *Pavlova*, ARA and EPA content decreased in both *Tisbe* and *Tachidius*, but DHA increased. However, both species contained the highest ARA-level of all treatments when feeding on *Pavlova* with 3.74 ± 0.95 ng µg C^{-1} in *Tisbe* and 4.86 ± 1.15 ng µg C^{-1} in *Tachidius*, respectively.

In *Tisbe,* the DHA:EPA-ratio ranged from 0.27 ± 0.05 (Ti-Phaeo) to 6.99 ± 1.34 (Ti-Iso) (Table 1.4). The DHA:EPA-ratio increased significantly from alga to copepod in Ti-Duna, Ti-Phaeo, Ti-Rhodo and Ti-Pav, but was decreasing in Ti-Iso (Table 1.5). In *Tachidius*, DHA:EPA-ratio ranged from 1.0 ± 0.24 (Ta-Pav) to 7.98 ± 1.88 (Ta-Duna). The ratio was significantly higher in *Tachidius* than in every diet. DHA:EPA-ratio increased more in *Tachidius* than in *Tisbe*, except for *Pavlova* as food source.

The ω3:ω6-ratio was significantly higher in *Tisbe* than in the diet, except for Ti-Phaeo, whereas *Tachidius* had a lower ω3:ω6-ratio than the corresponding diet.

Table 1.4: Fatty acid composition (ng µg C^{-1}) of *Tisbe sp.* (Ti) and *Tachidius discipes* (Ta) fed with five different algal species.
Rhodo = *Rhodomonas sp.*, Phaeo = *Phaeodactylum tricornutum*, Pav = *Pavlova sp.*, Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana*. Values are mean ± standard deviation, (N = 3, Ti-Phaeo: N = 2)

	Ti-Rhodo	Ta-Rhodo	Ti-Phaeo	Ta-Phaeo	Ti-Pav	Ta-Pav	Ti-Duna	Ta-Duna	Ti-Iso
C14:0	2.7 ± 0.2	5.0 ± 2.0*	10.4 ± 1.5	8.0 ± 1.8*	5.2 ± 0.8	7.2 ± 0.2	nd	3.1 ± 0.6*	5.3 ± 1.2*
C16:0	26.4 ± 2.9	28.9 ± 11.5	44.2 ± 13.2	50.1 ± 8.2	77.7 ± 15.2	93.7 ± 28.9	21.3 ± 8.3	58.7 ± 20.9	18.2 ± 2.7*
C16:1ω7+*iso*-17:0	6.1 ± 2.0*	nd[4]	71.4 ± 13.6*	6.0 ± 1.6	17.7 ± 8.4	10.9 ± 3.6*	5.0 ± 1.4*	6.2 ± 4.1	2.8 ± 2.7
C18:0	15.5 ± 4.3	17.0 ± 5.1	14.6 ± 4.3	29.5 ± 6.4*	44.1 ± 8.3*	49.0 ± 19.7	13.0 ± 5.6	30.4 ± 14.6	10.7 ± 2.6
C18:1ω9c	5.6 ± 1.3*	7.7 ± 5.0	18.3 ± 4.5*	7.2 ± 1.5	12.0 ± 3.5	14.5 ± 4.7	2.4 ± 0.3*	2.1 ± 1.3	9.7 ± 3.0*
C18:1ω7	5.6 ± 0.3	5.0 ± 2.0*	9.5 ± 0.2*	5.4 ± 1.0	nd*	nd	1.7 ± 1.2	8.8 ± 6.8	5.2 ± 0.6*
C18:2ω6c	2.7 ± 0.6	5.2 ± 3.6	4.4 ± 1.0*	nd*	5.7 ± 2.3	5.9 ± 1.2	1.1 ± 0.4*	nd*	3.7 ± 1.2*
C18:3ω6	nd*	7.5 ± 1.0*	0.61 ± 0.32*	13.6 ± 2.7	nd	0.60 ± 1.04	nd*	3.6 ± 0.5*	nd
C18:3ω3	12.7 ± 2.4	8.9 ± 7.5*	1.2 ± 0.1*	nd	4.7 ± 1.9	4.9 ± 2.0	4.5 ± 1.7*	3.1 ± 1.8*	7.4 ± 2.8
C18:4ω3	7.7 ± 0.9*	5.8 ± 4.0*	nd	nd	nd	nd	nd*	nd*	6.3 ± 1.2*
C20:4ω6	0.53 ± 0.13	1.3 ± 0.6	1.9 ± 0.1*	0.75 ± 0.15*	3.7 ± 1.0*	4.9 ± 1.2*	0.48 ± 0.12*	nd	nd*
C20:3ω3	nd	1.3 ± 0.6*	nd	0.55 ± 0.58	nd	3.3 ± 3.0	nd	1.3 ± 0.7	nd
C20:5ω3	13.2 ± 1.4	12.3 ± 6.2*	59.3 ± 4.4	9.0 ± 0.9*	17.2 ± 5.1*	13.7 ± 3.1*	3.3 ± 1.2*	0.52 ± 0.43	3.5 ± 1.4*
C22:6ω3	18.2 ± 5.1	31.0 ± 12.5	16.2 ± 4.3*	21.9 ± 1.0*	22.1 ± 7.9	14.0 ± 5.4*	11.4 ± 4.3*	3.7 ± 2.2	23.3 ± 4.3
total	120.8 ± 11.6	142.6 ± 61.4	278.7 ± 50.9*	156.9 ± 21.4*	215.2 ± 50.2	231.0 ± 35.5	65.3 ± 21.6*	125.2 ± 53.2	97.4 ± 18.5*
SFA[1]	45.5 ± 7.2	50.9 ± 17.8	72.4 ± 20.2	90.9 ± 16.7	128.4 ± 23.1	152.8 ± 49.3	35.0 ± 14.0	93.5 ± 36.6	35.0 ± 5.5*
MUFA[2]	14.4 ± 2.7*	13.3 ± 7.4*	121.3 ± 18.4*	14.6 ± 0.9*	31.5 ± 10.2	26.8 ± 10.6	7.6 ± 1.3	8.61 ± 5.78	13.9 ± 4.6*
PUFA[3]	47.9 ± 5.9*	73.3 ± 34.4	95.4 ± 10.8*	45.7 ± 4.4*	55.3 ± 17.2	51.8 ± 11.8	21.3 ± 7.6*	14.3 ± 5.2*	37.9 ± 9.5
DHA:EPA	1.4 ± 0.3*	2.6 ± 0.4*	0.27 ± 0.05*	2.4 ± 0.2*	1.3 ± 0.2*	1.0 ± 0.2*	3.4 ± 0.4*	8.0 ± 1.9*	7.0 ± 1.3*
EPA:ARA	26.3 ± 8.2	9.6 ± 0.8	30.7 ± 1.0*	12.2 ± 1.9*	4.6 ± 0.5*	2.8 ± 0.2*	6.8 ± 1.3*		
ω6	3.5 ± 0.7	14.0 ± 4.9	6.9 ± 1.3*	14.3 ± 2.8	11.4 ± 4.2	14.8 ± 2.5	1.6 ± 0.5*	5.6 ± 0.2*	3.7 ± 1.2*
ω3	51.8 ± 6.9	59.3 ± 29.5	76.6 ± 8.9*	31.4 ± 2.0*	43.9 ± 13.0	35.8 ± 8.3	19.3 ± 7.1*	8.6 ± 5.0*	40.5 ± 9.5
ω3:ω6	15.5 ± 4.9	4.1 ± 0.8	11.2 ± 0.9*	2.2 ± 0.4*	3.9 ± 0.3*	2.4 ± 0.3*	11.8 ± 1.4*	1.5 ± 0.8	11.2 ± 1.0*

* Superscript star denote significant differences between copepod and the corresponding algal species.
[1] Saturated fatty acids include additionally 12:0, 20:0, 22:0 and 24:0.
[2] Monounsaturated fatty acids include additionally 14:1, 15:1, 17:1, 18:1ω9t, 20:1ω9, 22:1ω9 and 24:1.
[3] Polyunsaturated fatty acids include additionally 16:2, 16:3, 16:4ω3, 18:2ω6t, 20:2ω6, 20:3ω6 and 22:2.
[4] nd = non detectable level

Table 1.5: Differences in fatty acid composition (ng µg C^{-1}) between the two copepods *Tisbe sp.* (Ti) and *Tachidius discipes* (Ta) and their food source in ng µg C^{-1}. Rhodo = *Rhodomonas sp.*, Phaeo = *Phaeodactylum tricornutum*, Pav = *Pavlova sp.*, Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana.* Values are mean ± standard deviation, (N = 3, Ti-Phaeo: N = 2)

	Ti-Rhodo	Ta-Rhodo	Ti-Phaeo	Ta-Phaeo	Ti-Pav	Ta-Pav	Ti-Duna	Ta-Duna	Ti-Iso
C14:0	-0.1 ± 0.2 [a]	-6.1 ± 2.0 [b]	2.4 ± 1.5 [a]	-15.1 ± 1.8 [b]	-1.2 ± 0.8 [a]	0.7 ± 0.2 [b]	-0.3 ± 0.0 [a]	2.3 ± 0.6 [b]	-26.9 ± 1.2
C16:0	1.3 ± 2.9 [a]	-11.8 ± 11.5 [a]	14.9 ± 13.2 [a]	0.4 ± 8.2 [a]	25.5 ± 15.2 [a]	41.4 ± 28.9 [a]	-8.2 ± 8.3 [a]	33.6 ± 20.9 [b]	-12.1 ± 2.7
C16:1ω7+*iso*-17:0	6.1 ± 2.0 [a]	-0.5 ± 1.3 [b]	52.4 ± 13.6 [a]	-64.6 ± 1.6 [b]	-6.2 ± 8.4 [a]	-13.0 ± 3.6 [a]	2.6 ± 1.4 [a]	4.1 ± 4.1 [a]	-0.2 ± 2.7
C18:0	3.0 ± 4.3 [a]	9.1 ± 5.1 [a]	-0.3 ± 4.3 [a]	26.5 ± 6.4 [b]	24.1 ± 8.3 [a]	29.0 ± 19.7 [a]	8.0 ± 5.6 [a]	20.9 ± 14.6 [a]	3.5 ± 2.6
C18:1ω9c	2.8 ± 1.3 [a]	0.1 ± 5.0 [a]	17.1 ± 4.5 [a]	2.0 ± 1.5 [b]	4.0 ± 3.5 [a]	6.5 ± 4.7 [a]	-2.1 ± 0.3 [a]	-1.8 ± 1.3 [a]	-18.6 ± 3.0
C18:1ω7	-8.3 ± 0.3 [a]	-10.4 ± 2.0 [a]	8.2 ± 0.2 [a]	3.7 ± 1.0 [b]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.5 ± 1.2 [a]	7.0 ± 6.8 [a]	3.2 ± 0.6
C18:2ω6c	-0.4 ± 0.6 [a]	-5.9 ± 3.6 [a]	3.0 ± 0.9 [a]	-5.6 ± 0.0 [b]	1.5 ± 2.3 [a]	1.7 ± 1.2 [a]	-4.3 ± 0.4 [a]	-5.1 ± 0.0 [b]	-7.8 ± 1.2
C18:3ω6	-0.3 ± 0.05 [a]	5.9 ± 1.0 [b]	0.6 ± 0.3 [a]	11.2 ± 2.7 [b]	0.0 ± 0.0 [a]	0.6 ± 1.0 [a]	-6.5 ± 0.0 [a]	-2.6 ± 0.5 [b]	0.0 ± 0.0
C18:3ω3	1.5 ± 2.4 [a]	-47.9 ± 7.5 [b]	1.2 ± 0.1 [a]	-0.4 ± 0.0 [b]	3.4 ± 1.9 [a]	3.5 ± 2.0 [a]	-41.9 ± 1.7 [a]	-32.3 ± 1.8 [b]	-0.1 ± 2.8
C18:4ω3	-9.8 ± 0.9 [a]	-42.4 ± 4.0 [b]	0.0 ± 0.0 [a]	-2.4 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	-1.8 ± 0.0 [a]	-2.5 ± 0.0 [a]	-5.9 ± 1.2
C20:4ω6	0.1 ± 0.1 [a]	0.2 ± 0.6 [a]	1.2 ± 0.1 [a]	-1.1 ± 0.2 [b]	-7.9 ± 1.0 [a]	-6.8 ± 1.2 [a]	0.5 ± 0.1 [a]	0.0 ± 0.0 [b]	-0.4 ± 0.0
C20:3ω3	0.0 ± 0.0 [a]	1.3 ± 0.6 [a]	0.0 ± 0.0 [a]	0.6 ± 0.6 [a]	0.0 ± 0.0 [a]	3.3 ± 3.0 [a]	0.0 ± 0.0 [a]	0.7 ± 0.7 [a]	0.0 ± 0.0
C20:5ω3	2.0 ± 1.4 [a]	-12.8 ± 6.2 [b]	7.7 ± 4.4 [a]	-65.8 ± 0.9 [b]	-25.7 ± 5.1 [a]	-29.2 ± 3.1 [a]	3.3 ± 1.2 [a]	0.5 ± 0.4 [b]	2.9 ± 1.4
C22:6ω3	10.6 ± 5.0 [a]	13.8 ± 12.5 [a]	14.1 ± 4.3 [a]	15.1 ± 1.0 [a]	20.8 ± 7.9 [a]	12.7 ± 5.4 [a]	11.4 ± 4.3 [a]	3.4 ± 2.2 [a]	6.5 ± 4.3
total	9.7 ± 11.6 [a]	-110.2 ± 61.4 [b]	135.0 ± 50.9 [a]	-120.0 ± 21.4 [b]	39.9 ± 50.2 [a]	55.6 ± 35.5 [a]	-59.3 ± 21.6 [a]	9.7 ± 53.2 [a]	-57.3 ± 18.5
SFA[1]	2.8 ± 7.2 [a]	-9.3 ± 17.8 [a]	19.3 ± 20.2 [a]	12.1 ± 16.0 [a]	47.4 ± 23.1 [a]	71.8 ± 49.3 [a]	0.0 ± 14.0 [a]	57.7 ± 36.6 [a]	-35.3 ± 5.5
MUFA[2]	3.3 ± 2.5 [a]	-17.6 ± 7.4 [b]	86.5 ± 18.4 [a]	-65.4 ± 0.9 [b]	-0.4 ± 10.2 [a]	-5.1 ± 10.6 [a]	0.7 ± 1.3 [a]	-1.1 ± 5.8 [a]	-19.4 ± 4.6
PUFA[3]	4.3 ± 6.5 [a]	-88.9 ± 34.4 [b]	26.3 ± 10.8 [a]	-72.9 ± 4.4 [b]	-7.1 ± 17.2 [a]	-10.7 ± 11.8 [a]	-57.9 ± 7.6 [a]	-55.7 ± 5.2 [a]	1.1 ± 9.5
DHA:EPA	0.7 ± 0.3 [a]	1.9 ± 0.4 [b]	0.23 ± 0.05 [a]	2.3 ± 0.2 [b]	1.2 ± 0.2 [a]	1.0 ± 0.2 [a]	3.4 ± 0.4 [a]	8.0 ± 1.9 [b]	-19.3 ± 1.3
EPA:ARA	0.7 ± 8.2 [a]	-23.1 ± 0.8 [b]	-37.7 ± 1.0 [a]	-29.8 ± 1.9 [b]	0.9 ± 0.5 [a]	-0.9 ± 0.2 [b]	6.8 ± 1.3 [a]	0.0 ± 0.0 [b]	-1.5 ± 0.0
ω6	-0.6 ± 0.7 [a]	-0.3 ± 4.9 [a]	4.8 ± 1.3 [a]	4.6 ± 2.8 [a]	-5.6 ± 4.2 [a]	-2.3 ± 2.5 [a]	-10.7 ± 0.5 [a]	-6.1 ± 0.2 [a]	-8.2 ± 1.2
ω3	4.3 ± 6.9 [a]	-88.0 ± 29.5 [b]	22.9 ± 8.9 [a]	-53.0 ± 2.0 [b]	-1.5 ± 13.0 [a]	-9.6 ± 8.3 [a]	-49.7 ± 7.1 [a]	-46.3 ± 5.0 [a]	3.4 ± 9.5
ω3:ω6	3.9 ± 4.9 [a]	-7.1 ± 0.8 [b]	-14.2 ± 0.9 [a]	-6.5 ± 0.4 [b]	1.2 ± 0.3 [a]	-0.3 ± 0.3 [b]	6.2 ± 1.4 [a]	-3.2 ± 0.8 [b]	8.2 ± 1.0

Dissimilar superscript letters denote significant differences between *Tisbe* and *Tachidius* fed with the same algal species

[1] Saturated fatty acids include additionally 12:0, 20:0, 22:0 and 24:0.

[2] Monounsaturated fatty acids include additionally 14:1, 15:1, 17:1, 18:1ω9t, 20:1ω9, 22:1ω9 and 24:1.

[3] Polyunsaturated fatty acids include additionally 16:2, 16:3, 16:4ω3, 18:2ω6t, 20:2ω6, 20:3ω6 and 22:2.

[4] nd = non detectable level

The MDS ordination indicates that *Dunaliella* was distinct from all other algal and copepod species (Figure 1.7). Several 70% similarity groups were formed. One group was composed of *Rhodomonas*, *Isochrysis* and the corresponding copepods. *Phaeodactylum*, *Pavlova* and the respective copepods formed another group, whereas *Dunaliella* was distinct from their corresponding copepods.

SIMPER analysis calculated the dissimilarities between groups and identified the fatty acids contributing the most to the differences. The lower level of 18:3ω3 and the higher level of DHA in Ti-Duna compared to *Dunaliella* were central to its dissimilarity of 39.64%. Whereas the lower level of 18:3ω3 and the higher level of 18:0 in Ta-Duna compared to *Dunaliella* were central to its dissimilarity of 38.41%.

Ti-Phaeo and Ta-Phaeo had a dissimilarity of 31.33%, with 16:1ω7+*iso*-17:0 and EPA contributing the most to that dissimilarity. *Phaeodactylum* and Ta-Phaeo had a dissimilarity of 34.57% with EPA contributing the most to the differences, whereas *Phaeodactylum* and Ti-Phaeo showed a dissimilarity of 20.16% with 16:1ω7+*iso*-17:0 contributing the most to the differences.
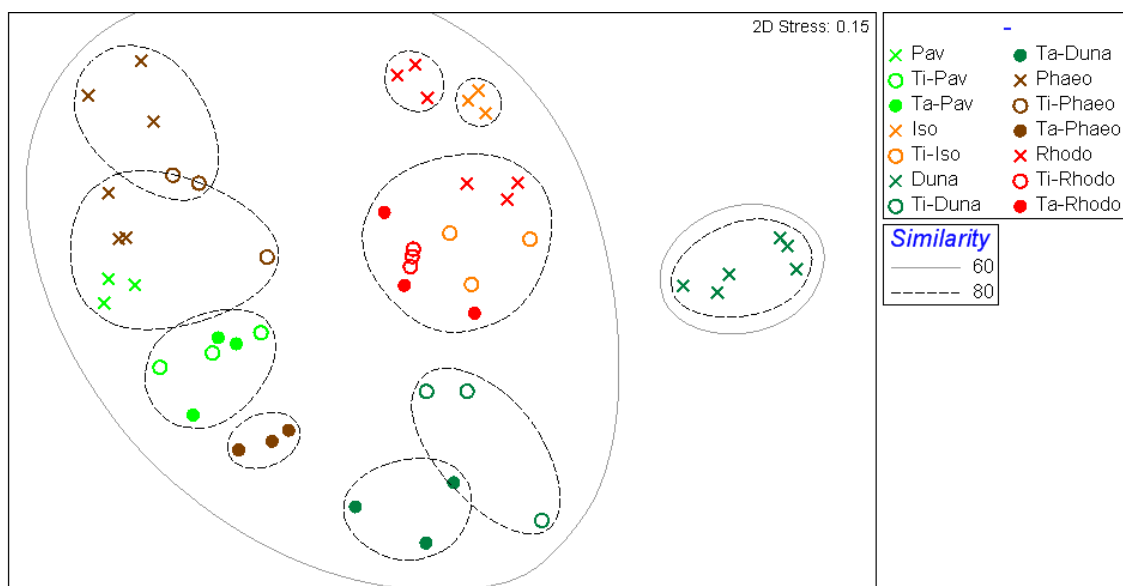
Figure 1.7: MDS-ordination with embedded Bray-Curtis-Similarity obtained by cluster analysis of the fatty acid profile of the different algal sources and the two copepods *Tisbe* sp. (Ti) and *Tachidius discipes* (Ta) fed with microalgae, N = 3, Ti-Phaeo N = 2. Similarity is expressed in percentage. The groupings marked by the dashed line represent 70% similarity and the solid lines represent 50% similarity based on cluster analysis. Rhodo = *Rhodomonas* sp., Phaeo = *Phaeodactylum tricornutum*, Pav = *Pavlova* sp., Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana.*

1.4. Discussion

1.4.1 Copepod performance

The choice of a desirable diet should be based on a short development time, high survival rate and high reproduction, which should be attained at a low diet concentration. With *Phaeodactylum* as the diet, both copepod species attained a high development index within 11 days at a low food concentration. However, the reproduction was slightly higher with *Rhodomonas* as the diet, but to obtain that, a higher food concentration was required. Compared to *Tisbe*, the survival of *Tachidius* was high also at low algal concentrations when fed with *Rhodomonas* or *Phaeodactylum* (Fig. 1.3). Consequently, *Tachidius* should be preferred over *Tisbe*. Since development time increased at lower rations (Fig. 1.1), the advantage of higher survival is offset. Furthermore, the same algal species differed in their absolute fatty acid contents at the time when the experiments with *Tisbe* and *Tachidius* were conducted (Appendix: Table A1 and A2). *Tisbe* generally got a lower amount of fatty acids in the case of *Phaeodactylum*, *Rhodomonas* and *Dunaliella*. This might be a reason for the lower survival at low algal concentrations compared to *Tachidius*.

However, the relative values of fatty acids in the diet were similar. Consequently, the conclusion that a high content in EPA (*Phaeodactylum*) or EPA and DHA (*Rhodomonas*) leads to high reproduction and a high growth performance can be made for both copepods, regardless of the different fatty acid contents of their diet.

While there is no conspicuous advantage of *Rhodomonas* over *Phaeodactylum* or vice versa indicated, *Dunaliella*, *Isochrysis* and *Pavlova* showed disadvantages in at least one of the parameters. Feeding with *Dunaliella* resulted in poor development of *Tachidius* and poor reproduction of both copepods. With *Pavlova* a 5-fold higher carbon concentration was needed to attain the same DI_{max} as with *Rhodomonas*. Since *Pavlova* is smaller than *Rhodomonas*, the higher saturation concentration can be explained by the observation of Frost (1972) that the carbon concentration at which the maximum ingestion rate occurs increases with decreasing cell size. However, *Isochrysis* also had a small cell size ($4.80 \pm 0.79 \, \mu m$) and *Tisbe* got food saturated at a quite low carbon concentration of $0.61 \, mg \, C \, L^{-1}$ and showed a high DI_{max} (6.84 ± 0.43), whereas *Tachidius* exhibited a slow and barely detectable development. For comparison, calanoid copepods respond with elongated generation times when fed with *Isochrysis* (Klein Breteler et al., 1995; Knuckey et al., 2005).

The different performances of *Tisbe* and *Tachidius* fed with *Isochrysis* were surprising. Possible explanations are: (a) *Tachidius* and *Tisbe* have a different feeding

apparatus, (b) *Tachidius* could not deal with the biochemical composition of *Isochrysis*, (c) *Tisbe* could use other food sources present in the culture vessel such as bacteria. Size seems not to be the decisive factor, since both copepod species could ingest *Pavlova* which was even smaller than *Isochrysis*, although requiring a higher food concentration than when fed with other algae. Thus, the way the copepods can deal with the biochemical composition of *Isochrysis* and possibly bacteria as additional food source seem to be explanations for the differences in development of the two copepod species.

Several studies showed that harpacticoids use bacteria as their food source (Decho and Moriarty, 1990; Guérin and Rieper-Kirchner, 1991; Perlmutter and Meyer, 1991; Dahms et al., 2007; De Troch et al., 2010). In the present study, the bacterial fatty acid biomarkers 16:1ω7, *iso*-17:0 (Desvilettes et al., 1997) were enriched ($+52.42 \pm 13.63$ ng µg C^{-1}, Table 1.5) in Ti-Phaeo compared to *Phaeodactylum* and were contributing the most to the differences in fatty acid composition between Ti-Phaeo and *Phaeodactylum*. The same occurred when *Tisbe* was fed with *Rhodomonas* but not to such an extent as with *Phaeodactylum*. This could be a hint for the use of bacterial food sources by *Tisbe,* although other fatty acid biomarkers of bacteria, like *iso*-15:0, 15:1 and others, were not present in *Tisbe*. Since our cultures have not been axenic, bacteria could have been utilized by *Tisbe*.

Harpacticoid copepods seem to need a higher algal concentration than calanoid copepods like *Acartia* spp., since Berggreen et al. (1988) and Knuckey et al. (2005) reported lower saturation concentrations, when fed with *Rhodomonas*, of 0.5 and 0.7 mg C L^{-1}, respectively. This might be due to different feeding behaviour. Calanoids are suspension-feeders (Paffenhöfer et al., 1982) whereas benthic harpacticoids graze on surfaces. To feed effectively they may require a dense algal coverage of the surface area rather than high algal concentrations in suspension.

The harpacticoid copepod *Euterpina acutifrons*, fed with a mixture of four different algal species, showed a comparable reproduction of 10.9 nauplii female^{-1} day^{-1} (Zurlini et al., 1978) like *Tisbe* and *Tachidius* in this study. *Acartia tonsa*, a calanoid copepod, likewise exhibited the highest fecundity with *Rhodomonas* (23.5 nauplii female^{-1} d^{-1}) (Peck and Holste, 2006), which is higher compared to our result. However, *A. clausii* was cultured in a 26-fold lower density, thus having a lower abundance of females that contribute to reproduction, which is leading to a lower overall production of calanoids. A lower total production also is observed in other mass production studies of calanoids (Støttrup et al., 1986; Payne and Rippingale, 2000) and harpacticoid copepods (Støttrup and Norsker, 1997; Rhodes, 2003).

In conclusion, *Tisbe* fed with the same diet like *Tachidius* showed the same or even better growth and reproduction performance, while getting lower absolute fatty acid contents. Consequently *Tisbe* might be a better candidate for aquaculture.

1.4.2 Transfer of fatty acids

Ti-Rhodo, Ti-Duna, Ti-Iso, Ta-Phaeo and Ta-Rhodo had similar EPA and DHA-contents on a percentage basis compared to wild zooplankton of temperate climates (Nanton and Castell, 1999). In comparison to cultured copepod species, the total fatty acid content of *Tisbe* and *Tachidius* fed with *Rhodomonas* and *Dunaliella* was similar to values observed in *Acartia tonsa*, although the EPA-content was higher in *A. tonsa* and the DHA-content differed slightly (Veloza et al., 2006).

The fatty acid compositions of *Tisbe* fed with *Isochrysis* and *Dunaliella* were similar to results of *Tisbe* investigated by Nanton and Castell (1999). However, Ti-Phaeo deviated especially in the EPA (21.9% of total fatty acid) and DHA-content (6.3%), but also in the 16:1ω7-content (23.5%) compared to *Tisbe* fed with *Chaetoceros calcitrans* (8.3, 21.4 and 0.4%, respectively), a diatom with similar fatty acid composition to *Phaeodactylum*. This again indicates the utilization of other food sources by *Tisbe* in the present study than the provided microalgae. De Troch et al. (2009; 2010) observed an increase in bacterial diversity on faecal pellets of a harpacticoid copepod than before in the diatom culture and concluded that bacteria function probably as an upgrade of the initial food source. The additional bacteria, probably originating from the copepod's digestive tract, depend largely on the initial food source. This explains why the utilization of bacteria was not observed with all the tested microalgal species in this study. Moreover, the harpacticoid copepod *Schizopera sp.* was able to reproduce and grow on an exclusively bacterial diet (Dahms et al., 2007). This all suggests that *Tisbe* used precursor fatty acids of bacterial origin to enhance its DHA-content, consequently the EPA-content of *Phaeodactylum* was conservatively incorporated, whereas *Tachidius* had to use the microalgal precursors such as 18:3ω3 and EPA to enhance its DHA-content.

The two copepod species showed different fatty acid patterns than their diet. Both copepods had a higher DHA-level and a higher DHA:EPA-ratio than their diet, except for Ti-Iso. *Isochrysis* already had a high DHA-level and therefore *Tisbe* did not seem to have the need for enriching this fatty acid. A percentage value of around 24% DHA is probably a saturation level for *Tisbe*, because even when fed with a mixed diet with a higher percentage of DHA, *Tisbe* did not exceed 26% DHA (Parrish et al., 2012).

Phaeodactylum and *Pavlova* had high values of EPA but low levels of DHA. The fatty acid composition of *Pavlova* sp. in this study deviated from other *Pavlova* species

(Volkman et al., 1991; Ponis et al., 2006). The DHA content of *Pavlova* in our study was lower, whereas the ARA content was higher. However, *Tachidius* was increasing the DHA content and decreasing the EPA content when fed with *Phaeodactylum* or *Pavlova*, while the 18:3ω3-level remained constant. Thus, the DHA:EPA-ratio increased.

Norsker and Støttrup (1994) tested the influence of three diets differing in their lipid and fatty acid content on the fecundity and fatty acid composition of *Tisbe holothuriae* and found an enrichment in EPA and DHA when fed with *Dunaliella*, but they also stated that the fatty acid composition of the diet was reflected to a large extent in the copepods. Their findings are in agreement with our finding that harpacticoids are able to produce EPA and DHA by elongation and desaturation of 18:3ω3 which is in high supply in *Dunaliella*. The same ability for elongation and desaturation was observed in a fresh water harpacticoid copepod (Caramujo et al., 2008). This shows that harpacticoids incorporate certain fatty acids in a conservative way if they are in the right amount and ratio to other fatty acids and if not they try to adjust the fatty acids to their needs. But their homeostatic ability is not perfect; otherwise all the treatments would result in a more or less similar fatty acid pattern, which is not the case as seen in Figure 1.6. *Tisbe* seems to have a higher desaturating capacity than *Tachidius*, which resulted in higher DHA and EPA values when having *Dunaliella* as a food source and also in a higher development index, 8.53 ± 0.23 compared to 2.93 ± 1.27, respectively. Nanton and Castell (1999) assumed a higher desaturating capacity for *Tisbe* sp. compared to *Amonardia* sp.

Although *Tisbe* achieved a good development when fed with *Dunaliella*, it had a very low reproductive output which could not be measured in our experiment due to a low number of females carrying an egg sac. The synthesis costs of DHA and EPA seem to be paid off by reduced reproduction. Norsker and Støttrup (1994) assumed that the synthesis of ω3-HUFAs might be rate-limiting for reproduction, because they also observed an enhancement in reproduction when fed with *Rhodomonas baltica* instead of *Dunaliella tertiolecta*.

The bioconversion in the ω6-chain, from 18:2ω6 to ARA, is less pronounced in the two copepod species than the ω3-conversion-chain, which is similar to other studies (De Troch et al., 2012; Parrish et al., 2012). *Tachidius* stopped the conversion process sometimes at 18:3ω6 and *Tisbe* only showed an increase when fed a diet low in ARA but high in EPA (*Phaeodactylum*) and when fed a diet without ARA (*Dunaliella*). When present at high concentrations in the diet (*Pavlova*) ARA was even reduced in the copepod. In conclusion, the desaturases were probably more active towards the ω3 fatty acid than the equivalent ω6 precursor, which also is the case in fish (Bell and Tocher,

2009) and the case of *Tisbe* showed that probably the ratio to other fatty acids such as EPA play a role.

1.4.3 Outlook: Copepods for fish larvae

Apart from other biochemical parameters like proteins, amino acids, pigments and vitamins, fatty acids play a central role in larval fish nutrition. The main focus is set on DHA, EPA and ARA-content and ratios between them. Reitan et al. (1994) eliminated mal-pigmentation of turbot when prey had a DHA:EPA ratio of 2:1 and Tocher and Sargent (1984) found this same ratio in several yolk sacs and eggs of marine fish larvae. Sargent et al. (1999a) suggested that an optimal DHA:EPA:ARA ratio for fish larvae would range around 10:5:1, but differing for each fish species. Further, they emphasised that both relative and absolute values of these fatty acids were important. The best larval development of *Sparus aurata* (Rodríguez et al., 1998) and the best pigmentation in *Scophtalmus maximus* larvae (Reitan et al., 1994) was obtained at a DHA-level of around 0.8% dry weight ($\approx$18.6 ng µg C^{-1}, converted with data of Øie et al. (1994)) in rotifers. This level is fulfilled by *Tachidius* when fed with *Rhodomonas* and *Phaeodactylum* with a DHA:EPA ratio of 2.62 ± 0.43 and 2.43 ± 0.15, respectively. *Tisbe* achieved 0.8% DHA when feeding on *Rhodomonas*, *Pavlova* or *Isochrysis*, but with lower or higher DHA:EPA ratios (1.36 ± 0.25, 1.27 ± 0.19, 6.99 ± 1.34, respectively) than suggested.

Ta-Phaeo and Ta-Rhodo had a DHA:EPA ratio of ca. 30:12:1 and 25:10:1, respectively, fulfilling the suggested DHA:EPA ratio but having a too high EPA:ARA ratio. When copepods where fed with *Pavlova*-concentrate they displayed a ratio of 6:5:1 (*Tisbe*) and 3:3:1 (*Tachidius*), respectively, having a better EPA:ARA ratio than with *Phaeodactylum* or *Rhodomonas* as a diet, but exhibiting a too low DHA:EPA ratio for optimal larval fish growth. A combination of *Rhodomonas* and *Pavlova*-concentrate as a diet could result in copepods which are optimal for rearing fish larvae, but the need for two algal species would lead to higher production costs.

In conclusion, an algal cell size of more than 5 µm is suitable for both copepod species. *Rhodomonas* or *Phaeodactylum* were best suited for copepod performance and these diets resulted, in terms of fatty acids, in copepods suitable for fish larvae. However, *Rhodomonas* is an algal species which is a bit demanding to culture due to sudden breakdowns or overgrowth by other algal species, also observed by Knuckey et al. (2005) and therefore it might be inappropriate for aquaculture purposes. This results in a recommendation of *Phaeodactylum* over *Rhodomonas*.

Acknowledgements

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Chapter 2

Evaluation of the suitability of benthic copepods as food for fish larvae and the potential digestibility of prey

Chapter 2

Evaluation of the suitability of benthic copepods as food for fish larvae and the potential digestibility of prey

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Abstract

This study aims to test the suitability of the harpacticoid copepod *Tachidius discipes* as an alternative live feed for fish larvae in comparison with the rotifer *Brachionus plicatilis*, a traditionally used live feed. Larvae of *Clupea harengus* were used as a model organism. *T. discipes* and *B. plicatilis* were offered as prey items, either as a sole diet or as a mixture of both. In order to investigate the suitability of the prey, RNA:DNA ratio, tryptic activity and growth of the fish larvae were determined.

The rotifer and mixed-treatment resulted in similar larval growth performance. In contrast, the majority of larvae, which were fed with copepods as a sole diet, died after 13 days. RNA:DNA ratio was significantly lower in this treatment compared to the other two treatments and the tryptic activity showed a decreasing trend. This compares well with starving larvae in other studies. However, the feeding incidence was significantly higher in copepod-fed larvae than in rotifer-fed larvae. This led to the assumption that they were not able to digest *T. discipes* sufficiently.

Based on this observation and conclusion, in-vitro evaluation of the digestibility of several potential prey organisms for larval fish was conducted using trypsin. *Artemia* sp. showed the highest degradation. *B. plicatilis* was less digestible than *Artemia* sp. The calanoid copepod *Acartia tonsa* and the harpacticoid copepod *Tisbe* sp. were more digestible than *T. discipes* and the nematode *Panagrolaimus* sp.

2.1. Introduction

Copepods are major natural food organisms for fish larvae. Calanoids are the dominant food source in the open marine water (Turner, 1984; 2004), whereas in coastal areas harpacticoids are an important link between primary producers and fish larvae (Sibert et al., 1977; Alheit and Scheibel, 1982). Both provide a good food quality (Shields et al., 1999; Evjemo et al., 2003), as well as an appetite stimulatory effect (Doi et al., 1997; Støttrup and Norsker, 1997). However, in artificial rearing of marine fish larvae, copepods are rarely used as the production of pelagic copepods requires a much higher effort than the production of *Artemia* nauplii. Therefore, the rearing of marine fish larvae relies mostly on rotifers and *Artemia*, which are relatively easy to culture, but both live feeds have deficiencies in their nutritional value compared to natural feeding organisms such as copepods. This results in weaker growth performance, higher mortalities or malpigmentation of fish larvae (Shields et al., 1999; Payne et al., 2001; Schipp, 2006; Busch et al., 2010). It is therefore natural to consider the replacement of the traditional live feed with copepods and to investigate their suitability as alternative live feed for fish larvae. This study deals specifically with the properties of harpacticoid copepods.

Benthic harpacticoids have some advantages compared to calanoid copepods: (1) they can be reared in higher densities than pelagic calanoid copepods (Støttrup, 2000), (2) harpacticoids are tolerant to salinity and temperature changes, and (3) they are able to feed on diverse food sources including microalgae, bacteria, organic matter and ciliates (McIntyre, 1969; Hicks and Coull, 1983). Hence, this study examined the suitability of harpacticoid copepods as feed for early stages of marine fish larvae. The results of Chapter 1 suggested *Tachidius discipes* as a suitable food source, based on their reproduction performance, their high fatty acid content of DHA (22:6ω3) and EPA (20:5ω3) as well as their DHA:EPA:ARA (ARA = 20:4ω6) ratio of 30:12:1 when fed with *Rhodomonas sp.*, which is close to the suggested ratio of 10:5:1 for marine fish larvae (Sargent et al., 1999a).

The nutritional condition of fish larvae can be monitored by the RNA:DNA ratio (long-term scale) and the tryptic activity (short-term scale) (Ueberschär and Clemmesen, 1992). Growth specifically in fish larvae means mainly protein synthesis. The theory behind the application of the RNA:DNA ratio is that the amount of DNA is constant in the cell even under starving conditions, whereas RNA is directly proportional to protein biosynthesis (Buckley, 1980). Thus, larvae in good nutritional condition have a higher RNA:DNA ratio than starving larvae. Trypsin is the major pancreatic enzyme to degrade protein in the early stages of fish larvae. It is secreted as its inactive form trypsinogen

from the pancreas into the intestine and is activated by enteropeptidase. The secretion is stimulated by the hormone cholecystokinin (CCK), which is released by chemical triggers in the intestine (Rønnestad et al., 2007). In humans, the presence of fats and proteins in the intestine is responsible for that stimulation (Chandra and Liddle, 2009). Consequently, the type and amount of food influences the trypsin concentration (Pedersen et al., 1987; Pedersen and Andersen, 1992). Thus, both indicators can be used in fish larvae to monitor the feeding activity, the food quality (Trypsin) and the nutritional condition (RNA/DNA, Trypsin) of fish larvae.

Feeding trials with herring larvae (*Clupea harengus*) comparing harpacticoid copepods (*T. discipes*) and rotifers (*B. plicatilis*) were performed to investigate the suitability of *T. discipes* as live feed for fish larvae and furthermore to test the suitability of *T. discipes* for mass culture. In order to evaluate the contribution of exogenous trypsin in the digestion process, which is controversially discussed (Dabrowski and Glogowski, 1977; Munilla-Moran et al., 1990; Zambonino Infante and Cahu, 1994; Ribeiro et al., 1999; França et al., 2010), the generic tryptic activity of the different prey types was analysed and related to the total tryptic activity measured in individual larvae.

Following the successful capture of prey its digestibility decides about the real nutritional value of the prey item. Thus, it is fundamental to know the digestibility when evaluating new prey items. Fleeger (2005) reported observations which suggest that harpacticoids may leave the gut of fish larvae alive and undigested. Moreover, interspecific differences of copepod digestibility occurred in turbot (Conway et al., 1993). Consequently, the digestibility of these types of copepods as first food for fish larvae needs to be evaluated. In order to compare with other live feed organisms, which are already applied in rearing of fish larvae, these prey items were included into the digestibility trial.

2.2. Material and Methods

2.2.1 Live feed mass culture

The harpacticoid copepod *Tachidius discipes* was collected from the Baltic Sea (Kiel Bay, Germany) in June 2009 and subsequently cultivated in the laboratory. The population was kept in flat plastic trays, providing a surface of 0.26 m^2 for the copepods, and filled with 7.8 L filtered sea water (FSW, 0.2 μm filtered, 17 PSU) at 18 ± 1°C with a 16 h:8 h-L:D-period. The copepod cultures were fed *Rhodomonas sp.* at food saturating levels, which resulted in good development and reproduction (Chapter 1). Copepods were maintained in a total of nine trays to feed the herring larvae. Daily, one to two trays

were used to harvest copepods for feeding the fish larvae. Accordingly, the same tray was harvested every 5 to 8 days. At the first two harvesting times, the size fraction 64 – 120 µm of the copepods was fed to fish larvae by using sieves with these mesh sizes. Subsequently the fraction 120 – 250 µm was harvested. This resulted in feeding nauplii (N) and copepodites (C) between stage N*IV* and C*III,* subsequently both nauplii and copepodites are referred to as copepods.

Rotifers were reared in a cylindrical tank with a conical bottom, filled with FSW and fed with resuspended *Nannochloropsis sp.* concentrate (BlueBiotech GmbH, Büsum, Germany). Prior to introducing individuals in the fish larval tanks, rotifers were enriched for 3h with S.presso (Selco, INVE Aquaculture, Belgium) in a small separate tank, following the suggested enrichment protocol provided with the product.

Artemia eggs (Premium Artemia, Sanders) were incubated for 24 hours in FSW at 30°C, harvested and the newly hatched nauplii were introduced into the fish larval tanks without any further treatment.

2.2.2 Larval feeding

To investigate the effect of different live feeds on the performance of herring larvae (*Clupea harengus*), the larvae were fed with *T. discipes* (copepods), *B. plicatilis* (rotifers) or a mixture (1:1) (mixed) of both for 30 days post hatch (dph). A total of nine 75 L-tanks were used, each filled with 30 L filtered Baltic Sea water (5 µm, UV-treated, 15 ± 1 PSU). The tanks were placed in one big squared tank filled with water for better temperature control. Temperature was kept constant at 11 ± 1°C and fish larvae were reared at a density of approx. 20 larvae L^{-1}. To exchange the water in the larval rearing tank, the water supply (5 µm-filtered, UV treated Baltic Sea water) was switched on for one hour prior to feeding (500 mL min^{-1}). The prey density was adjusted daily to 3 prey items ml^{-1}, by determining the prey density after the water exchange and adding prey items accordingly. At 18 dph all fish larvae, regardless of feeding treatment, were fed additionally *Artemia* sp. nauplii (1 nauplius mL^{-1}). From 23 dph on, all fish larvae were only fed with *Artemia* sp. nauplii (2-3 nauplii mL^{-1}). To analyse growth, RNA:DNA-ratio and tryptic activity, 20 fish larvae were sampled three h after adjustment of the prey density from each tank on 0, 4, 9, 12, 15, 18, 21, 24, 28 dph and subsequently stored in 1.5 mL plastic vials at -80°C until further treatment. The different food items were sampled out of the production tanks once at the end of the experiment and stored at -80°C for further fatty acid and tryptic activity analyses.

2.2.3 Digestibility of prey organisms

The digestibility of different prey organisms was investigated by incubating the organisms into a trypsin solution for 3 h. This time was chosen as a mean value of digestion time between 1.5 h (Fossum, 1983) and 5 h (Blaxter, 1965). Three food items (*T. discipes*, *B. plicatilis* and *Artemia* sp.) applied in the previous feeding experiment were used plus *Tisbe* sp. (Harpacticoida) and *Acartia tonsa* (Calanoida) in order to investigate differences between copepod orders. In addition, a candidate prey for larval rearing, the nematode *Panagrolaimus* sp. was included into the digestibility tests.

Trypsin (10 mg mL^{-1}) from bovine pancreas (1:250, SERVA GmbH, Germany) was solved in TRIS-buffer (0.1 M, pH 8) with $CaCl_2 \cdot H_2O$ (0.02 M). TRIS-buffer without trypsin was taken as a control treatment. The tryptic enzyme concentration was set significantly higher compared to the natural concentration in the gut of herring larvae, because no changes in the physical appearance were visible in preliminary tests when natural concentrations were chosen. The prey items were treated in two different ways in order to mimic the impact of the mechanical treatment of the ring muscles around the gut which may have an important contribution in the digestion of prey organisms: (1) prey items were not treated before introducing them in the trypsin solution and (2) prey items in a 1.5 mL vial were shock-frozen at -80°C for 2 min and then squeezed once with a sharp tweezer to imitate the possible damage of the prey item by the mechanical process of the larval ring muscles (Rønnestad et al., 2003).

Five prey items of each species were put in 1.5 mL vials filled with trypsin solution or buffer solution as a control. The vials were shaken on a shaking device (Mixer 5432, Eppendorf GmbH, Germany) for 3 h in a climate cabinet at 30°C. The temperature of 30°C was chosen, because bovine trypsin needs higher temperature than trypsin from cold adapted fish to show similar catalytic efficiency (Outzen et al., 1996). As a measure of the digestibility, photos of prey items were taken after 3 h with a microscope camera (AxioCam MRc, Zeiss GmbH, Germany) mounted on a microscope (Axio Observer.A1, Zeiss GmbH, Germany) and used to evaluate digestibility.

The total body area surrounded by the cuticle and the inner body area of the specimen in the control and the trypsin treatment were measured using ImageJ (v1.46r), in order to quantify the effect of trypsin solution on the prey organisms.

Subsequently, the percentage of inner body reduction (R) was calculated:

(1) $\qquad \overline{A_c} = \frac{1}{n} \sum_{i=0}^{n} (A_{ic}/A_{tc})$

(2) $\qquad R = 100 \cdot \left(1 - \frac{A_{it}/A_{tt}}{\overline{A_c}}\right)$,

where $\overline{A_c}$ = mean ratio of the inner body area (A_{ic}) and the total body area surrounded by the cuticle (A_{tc}) of the specimen in the control treatment, n = number of specimens, A_{it} = Area of the inner body of the specimen treated with trypsin, A_{tt} = total body area surrounded by the cuticle of the specimen treated with trypsin.

2.2.4 Analytical procedures

Growth and RNA:DNA-ratio

The standard length (SL) of thawed larvae and the percentage of larvae which had at least one prey item in their gut (feeding incidence) was noted prior to the analyses of RNA:DNA ratio and tryptic activity. Samples for RNA:DNA-ratio were then freeze dried for 24 h to a constant weight (Alpha1-4 freeze dryer, Christ GmbH, Germany) and subsequently weighed with a microbalance (SC2, Sartorius AG, Germany). The specific growth rate SGR (%) was calculated as:

(3) $\qquad SGR = \frac{(\ln W_{t1} - \ln W_{t0})}{N} \cdot 100$,

where $W(t_1)$ = weight at time t_1, $W(t_0)$ = weight at time t_0, N = number of time units between t_1 and t_0.

Analysis of RNA and DNA concentrations was performed with a modified method after Malzahn et al. (2003). The whole individual larva instead of only muscle tissue was analysed and subsequently the RNA:DNA-ratio was calculated of individual larvae.

Tryptic enzyme activity

Tryptic enzyme activity of individual fish larvae was assayed following the fluorescence-method described by Ueberschär (1995), with some modifications in order to fit the method to the application in microtiter plates: 250 µL substrate (Na-benzoyl-L-arginin-4-methylcoumarinyl-7-amid, Bachem AG, Switzerland) were added to 50 µL homogenate of the fish larva or prey organisms in a 96-well-plate. After mixing and an incubation time of 20 min (temperature adaptation), the relative fluorescence enhancement was recorded every 2 min for 12 min using a microtiter fluorescence reader (Fluoroskan Ascent, Labsystems Thermo). The tryptic enzyme activity is given as an equivalent of hydrolysed substrate per time unit (nmol hydrolysed substrate min^{-1} larva^{-1}).

<u>Fatty acid content of prey</u>

Fatty acids were extracted for 12 hours with a solvent mixture of chloroform:dichloromethane:methanol with a ratio 1:1:1. As internal standard, C13:0, C15:0, C17:0, C19:0 and C21:0 fatty acid methyl esters were added. C23:0-fatty acid was added for esterification control. After separation into an organic layer and an aqueous layer by adding a 1 M potassium chloride solution, sodium sulphate was added to the organic layer. After transferring the organic layer in a new glass cocoon, the fatty acids were converted to methyl esters at 50°C with a mixture of toluene and methanol which was supplemented with 1% concentrated sulphuric acid. The addition of 5% sodium chloride solution and hexane resulted in two layers. The hexane phase was transferred to a new glass cocoon and evaporated under reduced pressure. The extract was then redissolved with hexane to a final volume of 100 µL (modified after Christie, 1989).

The fatty acid methyl esters were analysed in a gas chromatograph (Trace GC-Ultra, Thermo Scientific Inc.) equipped with a flame ionization detector and a TR-FAME-column (10 m, 0.1 mm i.d., 0.20 µm film) with hydrogen as the carrier gas. The temperature programme started at 50°C for 1 min, increased by 30°C min^{-1} to 150°C, then 4°C min^{-1} to 180°C and 30°C min^{-1} to 240°C. Peaks were integrated using Chromcard software (Thermo Scientific Inc.) and identified with reference to known standards. The focus was set on ARA (arachidonic acid, 20:4ω6), EPA (eicosopentaenoic acid, 20:5ω3) and DHA (docosohexaenoic acid, 22:6ω3), but all fatty acids were included for the calculation of the total fatty acid content. Fatty acid values were biomass-normalized (ng FA µg C^{-1}).

To analyse the carbon content of *T. discipes* and *B. plicatilis*, approximately 150 organisms were filtered onto precombusted GF/F-filters (Whatman, 25 mm diameter), dried overnight and then analysed with an organic elemental analyser (FLASH 2000, Thermo Scientific Inc.).

2.2.5 Statistical analyses

Prior to statistical analyses, the assumptions of normality and homogeneity of variances were examined. The effects of the all three diets until 12 dph on the larval length, larval dry weight, SGR, tryptic activity and RNA:DNA-ratio of the larva were tested by a one-factorial-ANOVA, as well as the differences in the fatty acid content and tryptic activity of the prey. The effect of the mixed and the rotifer-treatment over the whole experimental time was analysed with a T-test (larval length, SGR, tryptic activity, RNA/DNA) and with a Mann-Whitney-U-test in the case of larval weight. Differences in

digestibility of the prey items were analysed by a two-factorial-ANOVA (prey type, pre-treatment). Post-hoc comparisons (Tukey HSD, $\alpha = 0.5$) were performed using STATISTICA 8. To analyse the effect of prey type on the feeding incidence, a Kruskal-Wallis-test with multiple comparisons of p-values was performed ($\alpha = 0.5$) with arcsin-transformed data. Unless otherwise stated, all values are presented as mean value ± standard deviation.

2.3. Results

2.3.1 Copepod mass culture

Overall, the harvest yields varied between trays (Fig. 2.1) and ranged from 10,486 (Tray C) to 39,577 nauplii and copepodites L^{-1} (Tray A) at the first harvesting time. The yield in all trays was decreasing over time and the harvest yields ranged from 2120 (Tray F and H) to 11,516 nauplii and copepodites L^{-1} (Tray A) at the respective last harvesting time.

The starting harvest yield was highest on tray A and was approximately 4-fold higher compared to tray I. Due to the high starting harvest yield, an average harvest yield of 4148 nauplii and copepodites L^{-1} day^{-1} was obtained. In contrast, the average harvest yields of all other trays with initial starting yields below 30,000 nauplii and copepodites L^{-1} (Tray B-I) was 1568 nauplii and copepodites L^{-1} day^{-1}.
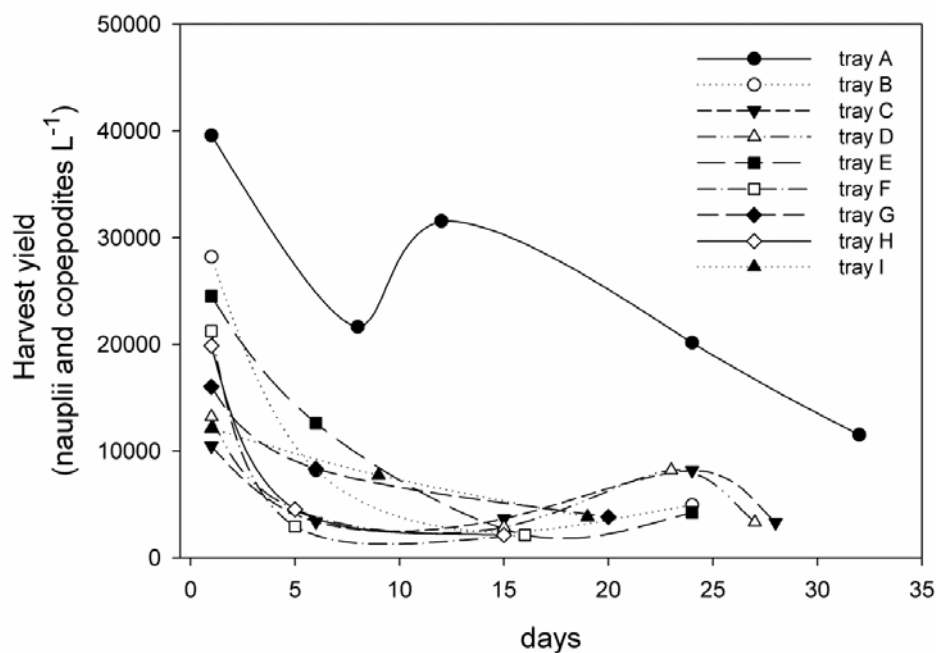
Figure 2.1: Harvested copepods of *Tachidius discipes* per litre during the feeding experiment. Each line represents one tray (tray volume: 7.2 L, surface: 0.26 m^2)

2.3.2 Growth rates of herring larvae

The majority of herring larvae fed with copepods died at 13 dph and therefore samples could only be collected until 12 dph for this diet to determine the standard length, dry weight and growth rate.

The standard length (SL) of the herring larvae was 6.9 ± 0.3 mm at hatching and increased almost linearly over 28 days (Fig. 2.2). For larvae fed with rotifers and a mixture of copepods and rotifers, the SL after 28 dph was 13.2 ± 2.3 mm and 12.9 ± 1.2 mm, respectively. The larvae fed with copepods showed an increase in larval length from day 9 to day 12. No significant differences could be found neither between all diets until day 12 (ANOVA: F = 1.64, p = 0.27) nor between rotifer and mixed-treatment until day 28 (T-test: t = -1.37, p = 0.11).
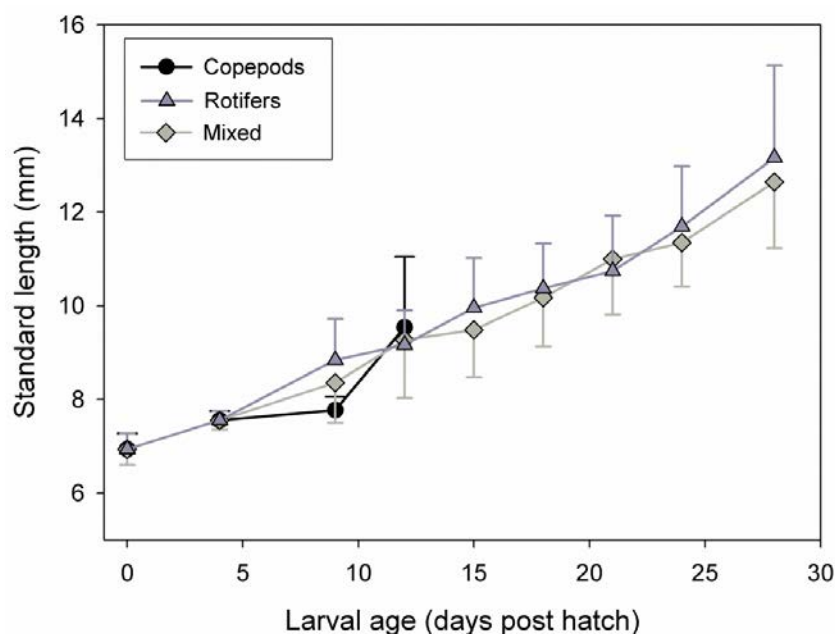
Figure 2.2: Standard length (mm) of *Clupea harengus*-larvae after hatching (dph) feeding on 3 different diets (copepods, rotifers, 1:1-mixture of both). Mean (N = 3) and standard deviation are displayed. From 18 dph (≈12.5 mm) the rotifer- and the mixed-treatment were supplemented with *Artemia* sp. From 23 dph *Artemia* sp. was the only food source.

Overall, the dry weight increased exponentially over 28 days (Fig. 2.3). However, first it decreased from 0 dph to 4 dph. The final dry weight was 79 ± 39 µg (copepods, 12 dph), 377 ± 159 µg (mixed, 28 dph) and 585 ± 533 µg (rotifer, 28 dph). The dry weight of herring larvae fed with rotifers scattered more compared to the mixed feeding regime. However, there was no statistically significant difference between the different diets (ANOVA: F = 3.8, p = 0.09, all diets until 12 dph; Mann-Whitney-U-test: U = 9, p = 0.08, rotifers and mixed until 28 dph).

Between 0 and 12 dph, the specific growth rates of larvae fed copepods, rotifers and a mixture thereof were 1.4 ± 0.9, 1.5 ± 1.3 and 2.1 ± 0.8% dry weight day^{-1}, respectively. The growth rate between day 0 and day 28 of rotifer- and mixed-fed larvae were 7.6 ± 1.5 and 6.2 ± 0.3% dry weight day^{-1}, respectively. The differences at both time intervals were not significant (ANOVA: F = 0.48, p=0.64 and T-test: t = -1.57, p = 0.19, respectively).
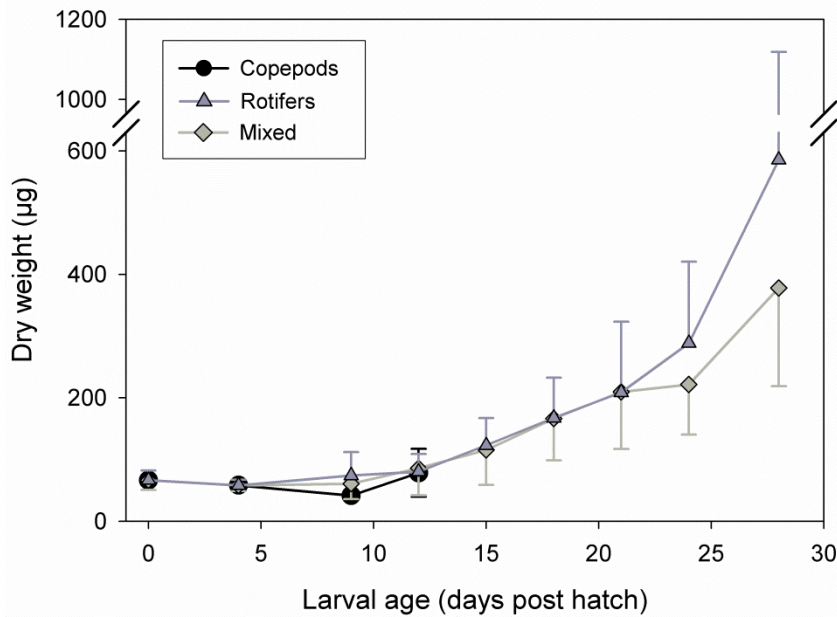

Figure 2.3: Dry weight (µg) of *Clupea harengus*-larvae after hatching (dph) feeding on 3 different diets (copepods, rotifers, 1:1-mixture of both). Mean (N = 3) and standard deviation are displayed. Feeding regime as described in Fig. 2.2.

2.3.3 Feeding incidence

The diet had a significant effect on the proportion of larvae which had at least one prey item in their gut (feeding incidence) (Kruskal-Wallis: H = 8.4, p < 0.05) (Fig. 2.4). Copepod-fed larvae had a significantly higher feeding incidence than rotifer-fed larvae (p < 0.05). The larvae fed with a mixture of both had a moderate feeding incidence, but was not significantly different to the other diets.
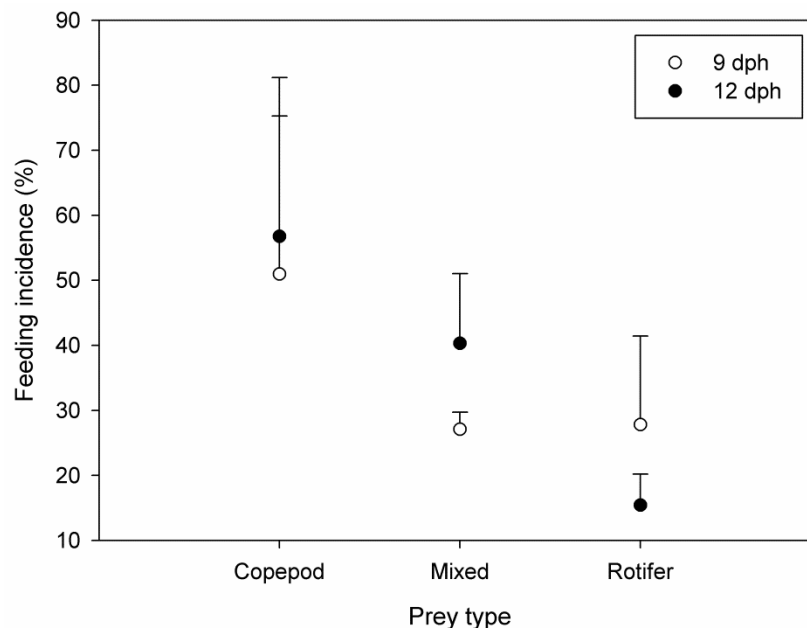
Figure 2.4: Proportion of larvae (*Clupea harengus*) which had at least one prey item in the gut (feeding incidence) in dependence of prey type at 9 and 12 dph. Mean (N = 3) and standard deviation are displayed.

2.3.4 RNA:DNA-ratio

The diet had a significant effect on the RNA:DNA ratio (ANOVA: F = 22.73, p < 0.001) (Fig. 2.5). The newly hatched larvae showed a ratio of 2.37 ± 0.10. The ratio of the herring larvae fed with rotifers and a mixture showed a decrease in the RNA:DNA ratio over the first four dph concomitant with the assimilation of the yolk sac. From 4 to 24 dph the ratio of the rotifer- and the mixed-treatment stabilised with a mean of 1.94 ± 0.15 and 1.89 ± 0.21, followed by a significant increase to a ratio of 2.79 ± 0.73 and 2.54 ± 0.56, respectively. The RNA:DNA-ratio of larvae fed with copepods decreased to 1.04 ± 0.13 at day 9, being significantly lower compared to the other two diets (p < 0.01). At day 13 most of the copepod-fed fish larvae died. No significant differences were observed between the rotifer- and the mixed-treatment (T-test: t = -1.05p = 0.35).
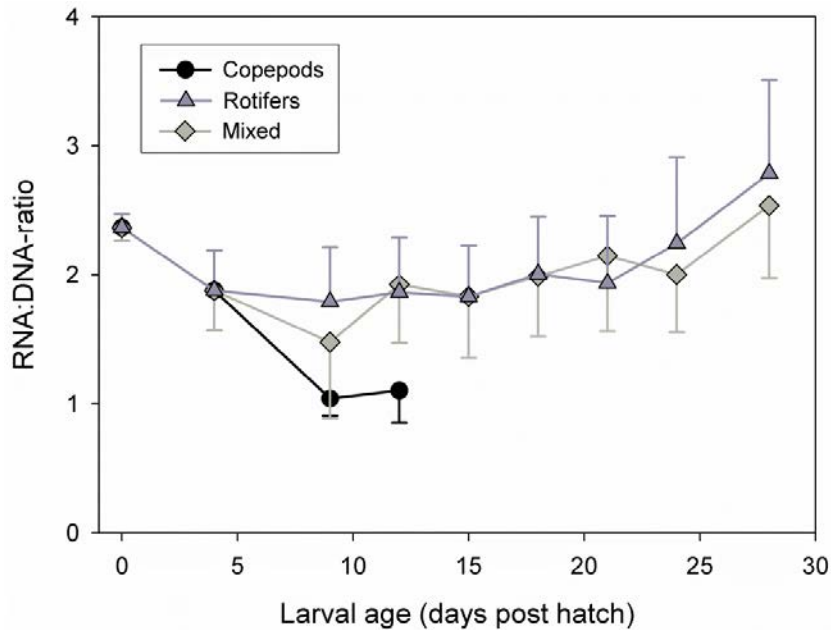
Figure 2.5: RNA:DNA-ratio of herring larvae (*C. harengus*), after hatching until day 28 (dph). The larvae were fed with 3 different diets (copepods, rotifers, 1:1-mixture of both). Mean (N = 3) and standard deviation are displayed. Feeding regime as described in Fig. 2.2.

2.3.5 Tryptic enzyme activity

The tryptic enzyme activity of the fish larvae showed high variability (Fig. 2.6) and there were no significant differences between treatments (ANOVA: F = 2.67, p = 0.15 all diets until 12 dph; T-test: t = 1.15, p = 0.31, rotifers and mixed until 28 dph). As a consequence only trends are described.

In general, the tryptic activity of herring larvae of all treatments decreased immediately after yolk sac absorption (≈ 7 mm length), regardless of the type of diet (Fig. 2.6). The enzyme activity of larvae of >8 mm increased with larval length when fed with rotifers and a mixed diet. This trend was more obvious in the mixed-treatment. However, when *Artemia* sp. was added to the diet 18 dph (≈ 12.5 mm larval length), the tryptic activity of the mixed-treatment and the rotifer-treatment decreased. Both treatment groups fluctuated around 1 nmol hydrolysed substrate min^{-1} larva^{-1}. The tryptic activity of fish larvae fed copepods decreased continuously until larvae were 11 mm in length. The subsequent increase to 2.1 nmol hydrolysed substrate min^{-1} larva^{-1} was just based on a measurement of a single fish larva.
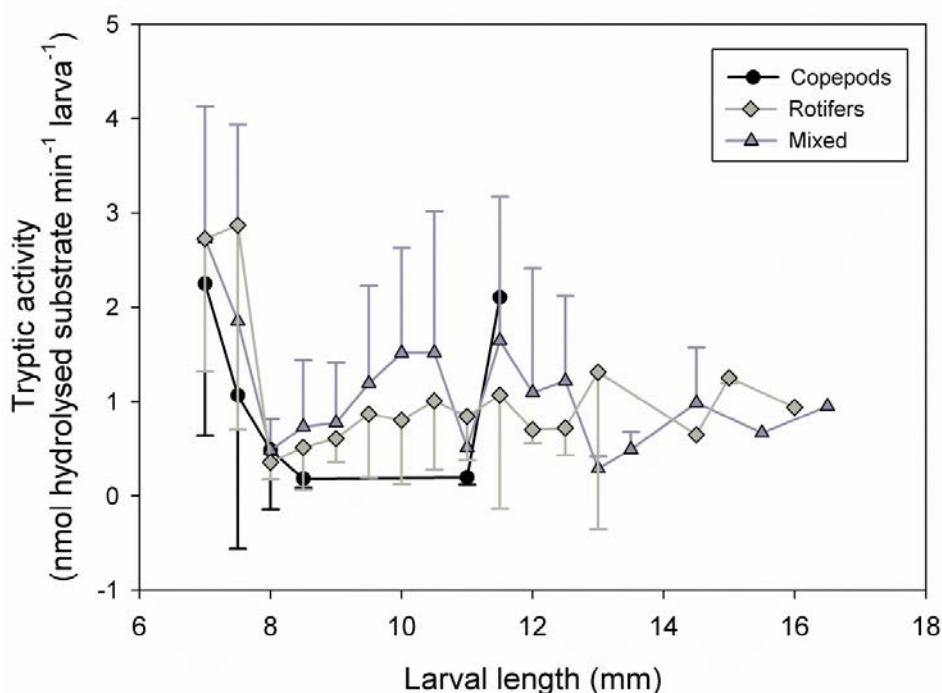
Figure 2.6: Tryptic activity per herring larva as a function of larval length (mm). The larvae were fed with 3 different diets (copepods, rotifers, 1:1-mixture of both). Mean and standard deviation are displayed. Feeding regime as described in Fig. 2.2.

The tryptic activity of the different prey items ranged from 0.011 ± 0.0004 (*T. discipes*) to 0.017 ± 0.005 nmol hydrolysed substrate min^{-1} (*Artemia* nauplii) (Table 2.1), with no significant differences between the prey items (ANOVA: $F = 2.72$, $p = 0.11$). The potential contribution of trypsin from an individual prey organism with a mean tryptic enzyme activity of 0.013 nmol hydrolysed substrate min^{-1} accounted for about 1.5% to the tryptic activity of an individual larva with a mean activity of 0.88 nmol hydrolysed substrate min^{-1}.

Table 2.1: Tryptic activity (nmol hydrolysed substrate min^{-1} $organism^{-1}$) of prey organisms (N = 3, each replicate consisted of N = 150, 100, 50, respectively). *Tachidius discipes* (size fraction 120-250 µm, approx. stages N*IV* to C*III*), enriched *Brachionus plicatilis* and newly hatched nauplii of *Artemia* sp.

Prey type	Mean tryptic activity ± SD (nmol hydrolysed substrate min^{-1} $organism^{-1}$)
Tachidius discipes	0.011 ± 0.0004
Brachionus plicatilis	0.015 ± 0.003
Artemia sp.	0.017 ± 0.005

2.3.6 Fatty acid content of prey

The fatty acid content differed between the three types of prey items (Fig. 7). The total fatty acid content was highest in *Artemia* sp. followed by enriched *B. plicatilis* and *T. discipes*. ARA was significantly higher in *B. plicatilis* than in *T. discipes* ($p < 0.05$). There was no significant difference in the content of EPA (ANOVA: $F = 2.58$, $p = 0.16$), whereas the DHA-content was significantly higher in *B. plicatilis* than in *Artemia* sp. and *T. discipes* (both, $p < 0.001$). As a result, the DHA:EPA ratio was significantly higher in *B. plicatilis* (2.35 ± 0.25) than in *T. discipes* (0.77 ± 0.12) and *Artemia* sp. (0.4 ± 0.2) (both, $p < 0.001$).
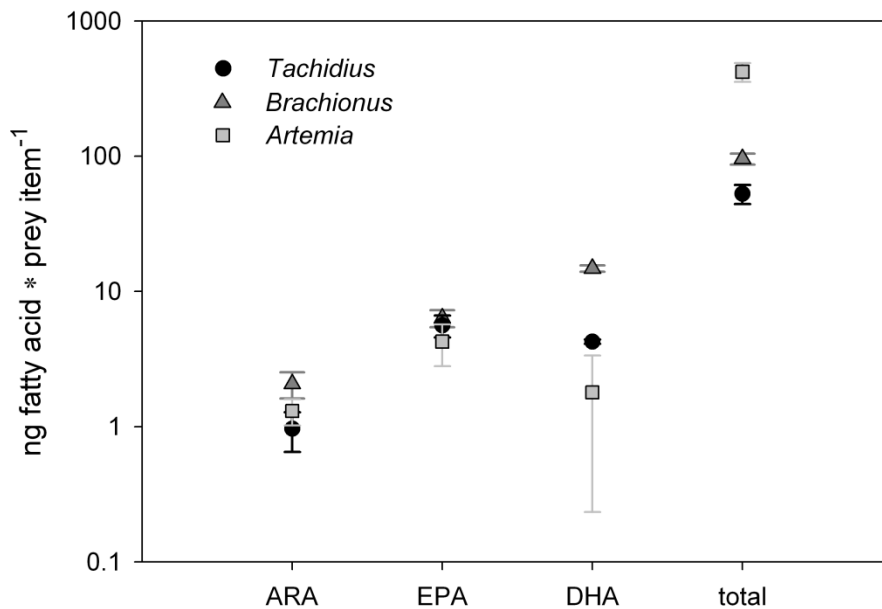
Figure 2.7: Fatty acid content (ng prey item[-1]) of enriched *Brachionus plicatilis, Tachidius discipes* (size fraction: 120 – 250 μm) and nauplii of *Artemia* sp. in log-scale. Mean (N = 3) and standard deviation are displayed.

2.3.7 Digestibility of prey organisms

The effect of trypsin on the different prey items is visualised with selected photos in the following figures below (Fig. 2.8 – 2.13).
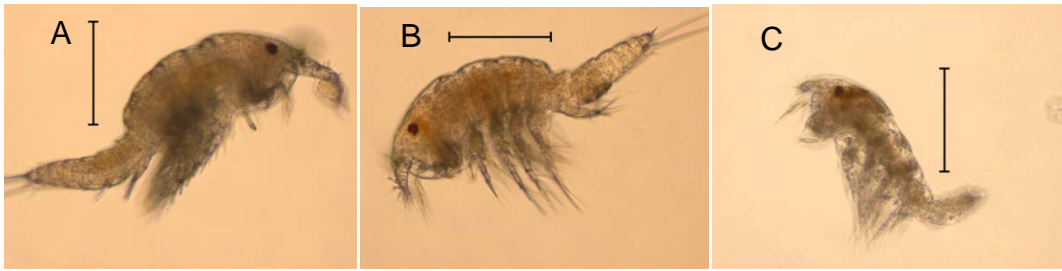
Figure 2.8: *Tachidius discipes* after 3 h A) in a TRIS-buffer (control), B) in a trypsin solution without squeezing and C) with squeezing, scale bar = 200 µm

Figure 2.9: *Tisbe* sp. after 3 h A) in a TRIS-buffer (control), B) in a trypsin solution without squeezing and C) with squeezing, scale bar = 200 µm
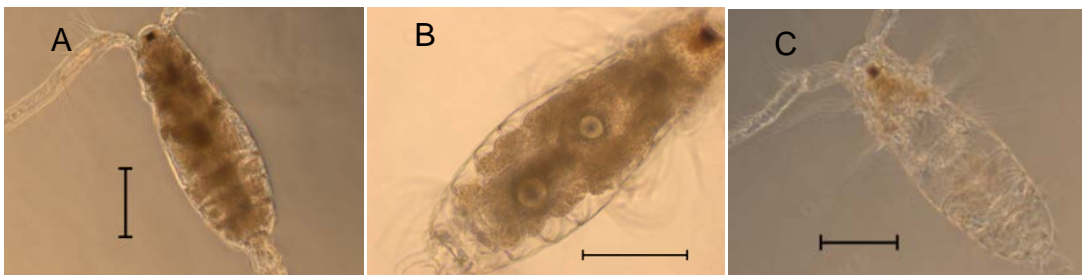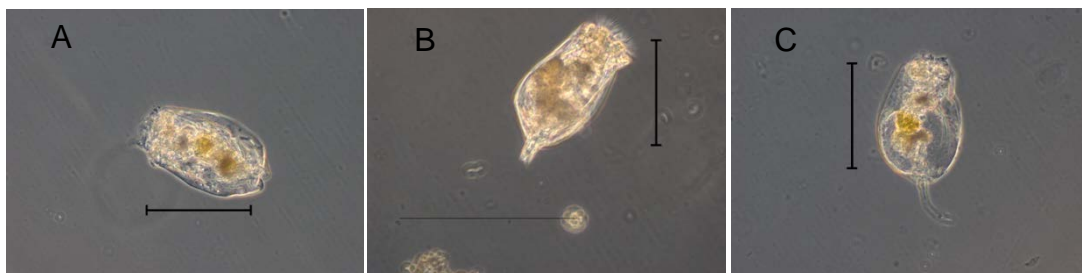

Figure 2.10: *Acartia tonsa* after 3 h A) in a TRIS-buffer (control), B) in a trypsin solution without squeezing and C) with squeezing, scale bar = 200 µm

Figure 2.11: *Brachionus plicatilis* after 3 h A) in a TRIS-buffer (control), B) in a trypsin solution without squeezing and C) with squeezing, scale bar = 200 µm
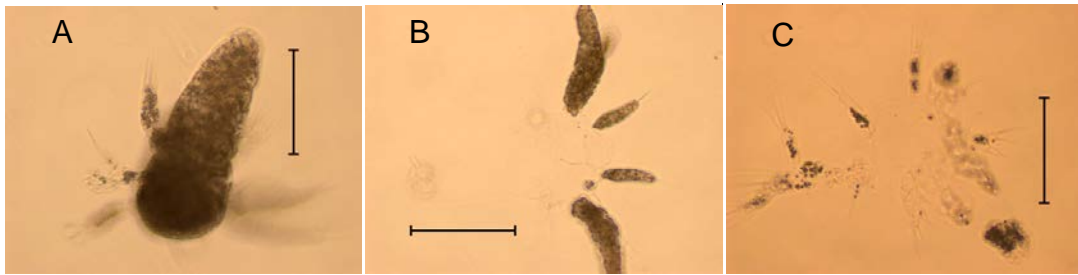
Figure 2.12: *Artemia* sp. after 3 h A) in a TRIS-buffer (control), B) in a trypsin solution without squeezing and C) with squeezing, scale bar = 200 µm

Figure 2.13: *Panagrolaimus* sp. after 3 h A) in a TRIS-buffer (control), B) in a trypsin solution without squeezing and C) with squeezing (only half of the nematode, because squeezing procedure led to a division in two parts), scale bar = 200 µm

After 3 h all prey items except of the nematode *Panagrolaimus* sp. were affected by trypsin, regardless of pre-treatment. The nematode just showed evidence of digestion if squeezed and a damage of the cuticle occurred, here division into two parts, prior to being treated with trypsin (Fig. 2.13C). Without squeezing they were still alive in the trypsin solution (Fig. 2.13B). The exoskeletons of the three copepod species were unharmed but the inner part of the body was obviously reduced compared to the control (Fig. 2.8 – 2.10). *A. tonsa* and *Tisbe* sp. showed a higher inner disintegration than *T. discipes*. *B. plicatilis* was partly still alive in the trypsin solution without being squeezed (Fig. 2.11). *Artemia* sp. was affected the most by trypsin (Fig. 2.12). Only parts of the antennae were left, but the thin cuticle was still visible.
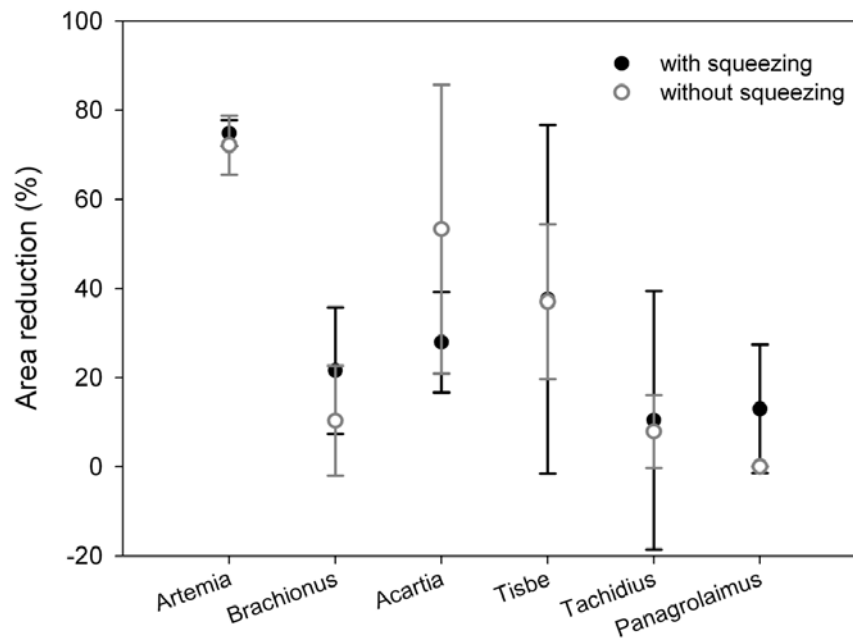
Fig. 2.14: Inner body reduction of different prey organisms after 3 h in a trypsin-solution (10 mg mL^{-1}, 30°C) compared to the control group (TRIS-buffer solution, 30°C). Mean (N = 3-5) and standard deviation are displayed.

Imitating the mastication process by squeezing the prey items ones before introducing them in the trypsin solution did not affect the digestibility of prey significantly (ANOVA: F = 0.06, p = 0.8) (Fig. 2.14). However, the digestibility differed significantly between prey types (ANOVA: F = 10.99, p < 0.001). *Artemia* sp. exhibited the highest digestibility, which was significantly higher than the digestibility of *T. discipes* (p < 0.001), *B. plicatilis* (p < 0.01) and *Panagrolaimus* sp. (p < 0.001). Furthermore, differences between copepod species were observed. *A. tonsa* the calanoid copepod and *Tisbe* sp., one of the harpacticoids, were significantly more digestible than the harpacticoid copepod *T. discipes* (p < 0.01 and p < 0.05, respectively). *T. discipes* and the nematode *Panagrolaimus* sp. were least affected by the treatments.

It has been observed once during the experiment that a harpacticoid copepod nauplius can survive the passage through the larval gut (Fig. 2.15). The individual left the gut of a 12 day old larva unharmed and alive.
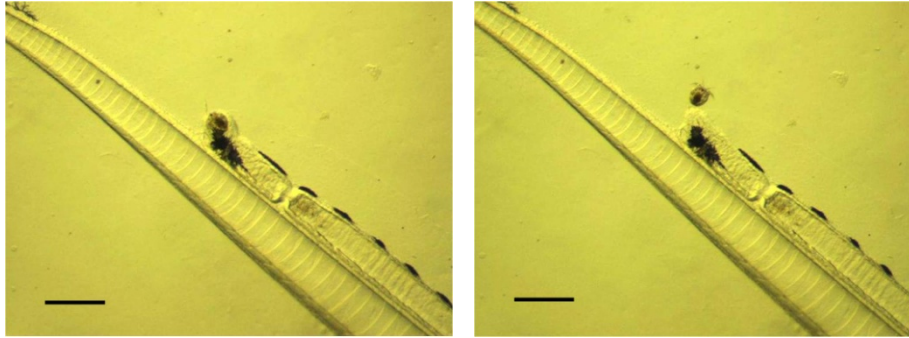
Figure 2.15: Nauplius of *Tachidius discipes* excreted from the gut of a 12 day old larva (*Clupea harengus*), apparently unharmed and alive. Scale bar = 300 µm.

2.4 Discussion

The present study examined the option for mass cultivation of *T. discipes* and its nutritional value and digestibility as live feed for fish larvae.

2.4.1 Suitability of harpacticoid copepods

The starting density in the trays was too low to sustainably harvest copepods every 5 – 8 days and consequently the harvesting yield decreased over time. Using trays with high starting densities could be feasible as demonstrated in this study (Tray A) and a sustainable production is basically possible. A further approach to sustainability by increasing productivity is the use of a copepod culture dominated by adult females (Støttrup and Norsker, 1997). In general, the batch culture of harpacticoid copepods results in relatively high productivity in comparison to calanoid copepod culture systems (Payne and Rippingale, 2001). However, a continuous culture system should be considered for the up-scaling of cultures (Støttrup and Norsker, 1997).

The tryptic activity of the fish larvae in these experiments showed in general a high variability. This is probably due to varying individual trypsinogen syntheses in the pancreas (Pedersen and Andersen, 1992), which might be a result of different feeding conditions and general performance of the individual fish larvae. Nevertheless, the tryptic activity, a short-term indicator of food quality and nutritional status, followed in these experiments a previously described pattern, where the digestive enzyme capacity changed with larval age in four phases along the ontogenetic development of fish larvae (Pedersen et al., 1987; Ueberschär, 2006). Due to sampling interval and period only phase II and III were represented in this study. These phases are characterised by a decline of tryptic activity in the first feeding stages until a larval length of 7.5 mm, which

probably can be attributed to an ontogenetic deficiency to balance the demand of trypsin and the trypsinogen synthesis (Pedersen et al., 1987; Ueberschär, 2006) and was followed subsequently by phase III characterized by an increase of the tryptic activity. The reason for the decrease in phase II is still to be verified and needs further in-depth study (Rønnestad et al., 2013).

The larval tryptic activity of copepod-fed larvae did not show an increase in phase III, but remained low instead. Furthermore, a high mortality and a decrease in RNA:DNA ratio occurred in copepod-fed fish larvae at 13 dph. This compares well with Pedersen et al. (1987), where starvation of *C. harengus* resulted in high mortality and behavioural signs of starvation at 13 dph. Thus, the herring larvae fed harpacticoid copepods were obviously starving although they had a significantly higher feeding incidence than rotifer-fed larvae. The increased feeding activity or appetite was probably induced by movements of copepods (Doi et al., 1997; Støttrup and Norsker, 1997) or chemical stimuli (Dempsey, 1978), but it did not result in better growth performance of the herring larvae.

This low performance can have two causes: (1) the nutritional value of the copepods (e.g. caloric content and fatty acids) was not sufficient to support growth of the fish larvae or (2) herring larvae reveal a weak digestibility of harpacticoid copepods.

The caloric content of rotifers (0.0036 J ind^{-1}) is 2.4 times larger compared to *Tisbe* nauplii (0.00147 J ind^{-1}) (Støttrup and Norsker, 1997). In this study, bigger naupliar stages and first copepodite stages were used. Accordingly, the energetic difference between the rotifers and the copepod stages should be small or negligible. *Artemia* sp. with a lower DHA content than *T. discipes* proved to be an appropriate diet for herring larvae. Thus, the lower DHA content of *T. discipes* in comparison to *B. plicatilis* in the present study cannot be the reason for the poor performance of copepod-fed larvae.

It is suggested that the digestibility of harpacticoid copepods is obviously a major issue. If the prey item shows a low digestibility, bio-molecules like proteins, amino acids and fatty acids, which trigger the pancreatic enzyme secretion (Liddle, 2000; Chandra and Liddle, 2009), cannot be digested efficiently and hence their absorption is low. Consequently, the tryptic activity remains low, like in this study.

Comparing the mixed with the rotifer-treatment, no statistical differences in growth, RNA:DNA ratio and trypsin activity were observed. The initial decrease in RNA:DNA ratio is similar to observations made by Clemmesen (1987; 1994) and is probably caused due to changes from endogenous to exogenous feeding concomitant with physiological changes. Since RNA:DNA ratios of fish larvae reflect the feeding environment faced about 4 days before sampling (Clemmesen, 1994), the significant increase at day 28 results from the change to *Artemia* nauplii as the sole diet from day 23 onwards. This is

probably due to the fact that *Artemia* sp. (224 J mg^{-1} dry weight (DW), (Dhont and Van Stappen, 2003)) provides a higher energy content than *B. plicatilis* (18.5 J mg^{-1} DW) and *T. discipes* (20.5 J mg^{-1} DW, for *Tigriopus californicus*) (Theilacker and Kimball, 1984), resulting in a higher growth rate, which is also reflected in the larval RNA:DNA ratio.

The contribution of exogenous enzyme activity has been controversially discussed in the past (Dabrowski and Glogowski, 1977; Munilla-Moran et al., 1990; Zambonino Infante and Cahu, 1994; Ribeiro et al., 1999; França et al., 2010). The results of the present study demonstrated that the exogenous enzyme activity had a minor contribution to the total tryptic activity measured in individual larvae. The ingested prey contributes with only ~1.5% to the total mean tryptic activity of 0.88 nmol hydrolysed substrate min^{-1} larva^{-1} without any significant differences between the types of prey.

2.4.2 Digestibility of prey

Our study provided strong evidence for a low digestibility of *T. discipes*. In order to be able to quantify the digestibility of *T. discipes* in larval guts in comparison to common live feed in aquaculture, we performed, to the best of our knowledge, the first time an in vitro digestibility test with *T. discipes*, compared to a number of other common and potential live feed organisms for fish larvae.

Although other enzymes, such as amylase, lipase and phosphatase, contribute to the digestion of prey items in first feeding fish larvae, trypsin is a key enzyme in larval intestines (Pedersen and Andersen, 1992). Even though having used a trypsin concentration beyond a natural concentration in order to achieve a visible outcome, the results are being considered as useful. However, it should be noted that the natural orchestration of the various enzymes participating in larval digestion are probably more efficient compared to the conditions applied in this in-vitro experiment. Nevertheless, this study provides a first indication about the digestibility of different prey items, but not a full account of all steps involved in the digestion process.

The imitation of the mastication process described by Rønnestad et al. (2003) had no significant effect on the digestibility. However, the tested nematode only showed an evidence of digestion if the cuticle was damaged previously. Walford and Lam (1993) stated that damage of rotifers is necessary to cause an autolysis and suggested that pharyngeal teeth may perform this function in sea bass larvae. Thus, a previous damage seems to be necessary to digest the prey organisms effectively. The singular squeezing in the present study might not have been an appropriate imitation of the mechanical process in the fish larva. Nevertheless, whether the two processes in the larva described above are capable to damage the flexible, thin nematode needs further investigation.

Compared to other prey items including *Tisbe* sp. likewise a harpacticoid copepod, *T. discipes* showed a low digestibility, which is according to the observation of a live gut passage by a nauplius of *T. discipes* and the poor growth performance of copepod-fed larvae in the feeding experiment. Interspecific differences in digestibility between copepods were also observed by Conway et al. (1993). Additionally, herring larvae can be successfully reared with *A. tonsa* (Pedersen et al., 1987) and turbot larvae with *Tisbe holothuriae* (Støttrup and Norsker, 1997). The digestibility of those two copepod species was higher compared to *T. discipes*. Thus, larvae of *C. harengus* have sufficient mechanical means and enzyme activity to digest calanoid copepods but not to digest the harpacticoid copepod, *T. discipes*, which is apparently more difficult to break down.

The differences in the digestibility of prey items may result from the different structures of the cuticle of the tested prey items. Bresciani (1986) found a remarkable variation of the cuticular fine structure in copepods. The copepod cuticle consists of a protein matrix with lipids and rods of chitin (Bouligand and Neville, 1973 in (Boxshall, 1991)). Although the cuticle is not digested by fish larvae, it is segmented which in turn allows enzymes to penetrate into the inner soft tissue of the prey. The different digestibility of the two harpacticoids is supposed to be a consequence of their different natural habitats. *T. discipes* was isolated from a coarse sand at the eulittoral, whereas *Tisbe* sp. is found more often in the phytal (Hicks, 1980). *T. discipes* might need a stiffer exoskeleton and segments which are closer connected to resist the turbulences and swirling sand grains than *Tisbe* sp. Calanoid copepods, such as the tested species *A. tonsa*, require a low weight to be able to float in the pelagial and to minimise the energetic costs for maintaining a certain depth, which results in a more fragile exoskeleton than harpacticoids.

In contrast to copepods, the cuticle of rotifers contains neither chitin nor collagen and has no skeletal function (Clément and Wurdak, 1991). The underlying integument is an intracytoplasmic lamina, which is thicker around the trunk and contains keratin like proteins. This lamina is stiffened by disulphide bridges, which cannot be dissolved by proteolytic enzymes such as trypsin. However, other soft parts of the body were digested by enzymes (Bender and Kleinow, 1988). Thus, a high digestibility would have been expected. *B. plicatilis* can have a relatively high carbohydrate content of around 20 % of dry weight (Frolov et al., 1991) compared to *Artemia* sp. (10.6%) (Léger et al., 1986) or copepods (0.2 – 5.1%) (Båmstedt, 1986). Consequently, the interaction of all enzymes, especially amylase in this case, plays probably a bigger role for the digestion of rotifers than for the other tested prey organisms.

Newly hatched *Artemia* nauplii (Branchiopoda) have a very thin cuticle with 0.3 – 1.0 μm thickness (Freeman, 1989). Furthermore, the procuticle is not yet differentiated

into an endo- and exocuticle and is not calcified (Martin, 1991). As a consequence, the enzyme solution can obviously penetrate into these nauplii and digest the inner part.

The cuticle of nematodes has a flexible multi-layered structure (Poinar Jr., 2001). It contains protein and collagen (Watson, 1965) and is not segmented (Wright, 1991). Therefore, the enzyme equipment available in the early stages of fish larvae might not be able to penetrate this structure. Consequently, trypsin cannot enter the nematode to digest the inner part unless a damage of the cuticle occurred previously.

2.5 Conclusion

The batch culture system for copepod production in this study was apparently not sufficient to achieve a sustainable harvest rate. The continuous system operated by Støttrup and Norsker (1997) presents a promising approach but deficiencies in productivity still remain. However, even low numbers of copepods added to the standard feeding protocol results in enhanced growth performance and pigmentation (Doi et al., 1997; Heath and Moore, 1997; Olivotto et al., 2008b). Thus, a supplementation or a feeding at a short time period with copepods would reduce the required number of copepods and would facilitate their sufficient provision.

The low performance of herring larvae fed with *T. discipes*, together with a low tryptic activity and a low RNA/DNA-ratio as well as the subsequent digestibility test revealed that herring larvae were obviously not able to digest this copepod species sufficiently. The interspecific differences in the digestibility of *T. discipes* and *Tisbe* sp. and their respective habitats lead to the assumption that robustness is to the expense of their digestibility. Consequently, besides the nutritional value such as fatty acid composition and protein content of the food organism, the accessibility to these nutrients is an important criterion when evaluating a new prey type.

Chapter 3

Providing harpacticoid copepods via floating sieve improves fish larval feeding success

Chapter 3

Providing harpacticoid copepods via floating sieve improves fish larval feeding success

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Keywords

Foraging behaviour, video analysis, live feed, rotifer, herring fish larvae, *Clupea harengus*

Abstract

We compared the swimming behaviour and feeding success of herring larvae (*Clupea harengus*) in the presence of harpacticoid copepods (*Tisbe* sp. and *Tachidius discipes*) and rotifers (*Brachionus plicatilis*), because harpacticoid copepods are being considered as alternative candidates for live feed in aquaculture. The comparison was performed at 5 and 10 days post hatch (dph) via 2D-video observations. We also investigated the potential advantage of feeding *Tachidius* via a floating sieve because the orientation of harpacticoids towards the bottom of the tank may pose problems for pelagic food searching fish larvae. Quantitative analyses of larval trajectories allowed the estimation of feeding behaviour through a series of indicators: swimming speed, straightness of the trajectories, turning angles and swimming activities (break, sink, slow, normal, and fast). The outcomes highlighted that the prey type had no significant effect on swimming speed or straightness of the swimming path. However, at 10 dph directly copepod-fed larvae spent less time in slow but more time in the normal swimming-state than rotifer-fed larvae and larvae fed with *Tachidius* via a sieve. This suggests higher energy expenditure of directly copepod-fed larvae. In addition, the feeding success was higher in larvae fed with *Tachidius* via sieve than directly *Tachidius*-fed larvae. Furthermore, *Tisbe* was obviously easier to capture than *Tachidius*, because *Tisbe*-fed larvae exhibited a higher feeding success than *Tachidius*-fed larvae. In conclusion, providing harpacticoid copepods via a floating sieve can improve the rearing of marine fish larvae and moreover, *Tisbe* should be preferred over *Tachidius* as a food organism.

Chapter 3

3.1. Introduction

Rotifers are easy-to-rear and consequently used as a common live food species in aquaculture, but are often of suboptimal food quality for fish (Payne et al., 2001; Busch et al., 2010). Copepods are known to improve survival, growth and development of fish larvae by increasing the food quality (Shields et al., 1999). Harpacticoid copepods are in particular a potential food in aquaculture to feed fish larvae (Støttrup and Norsker, 1997). Furthermore, they have advantages over calanoid copepods, including the utilization of diverse food sources (McIntyre, 1969) and the tolerance of higher culture densities (Støttrup, 2000).

However, not only cultivation and quality of the prey organisms are important but also the ability of the fish larvae to capture them. The capture success of fish larvae is influenced by prey escape response (Buskey et al., 1993; Titelman and Kiørboe, 2003), prey swimming behaviour (Viitasalo et al., 1998) and prey visibility (Eggers, 1977). The predator avoidance efficiency is strongly depending on both, prey life stage (Fields and Yen, 1997; Titelman, 2001) and species (Fields and Yen, 1997; Buskey et al., 2002).

In general, rotifers exhibit a slow cruising movement, whereas copepods display a more elusive swimming behaviour and show a predator avoidance mechanism by performing fast escapes in response to hydromechanical signals caused by predators (Kiørboe and Visser, 1999; Strickler and Balázsi, 2007; Waggett and Buskey, 2007). Therefore, rotifers are easier to catch because of their slower movement. First-feeding cod larvae, for example, preferred slow swimming protozoa over calanoid copepod nauplii (Hunt von Herbing and Gallager, 2000). On the other hand, the copepods' movement is considered to be stimulating for the fish larvae (Heath and Moore, 1997). In this context, especially harpacticoid copepods might be advantageous due to their weaker escape abilities compared to calanoids (Beck and Turingan, 2007), which can lead to a higher capture success in fish larvae. At the same time, their orientation towards the bottom of the tank is potentially posing a problem for food searching fish larvae, since no perception of particles occurs when being underneath or high above the larva (Rosenthal and Hempel, 1970). It is therefore of interest for the aquaculture industry whether harpacticoid copepods are sufficiently available for fish larvae which sometimes are poor predators at the first days of their feeding phase, because of their limited sensory capability and manoeuvrability (Chesney, 2007).

To overcome the potential obstacle for larvae of low prey availability, harpacticoid copepods might be cultured in a floating sieve directly at the larval rearing tank described by Kahan et al. (1982). Nauplii and early copepodite stages of harpacticoids can pass

through the sieve and are directly available for the fish larvae. As a consequence, the younger copepod stages probably stay a longer time in the water column and fish larvae may benefit from this type of food supply in terms of perception and energy demand.

Herring larvae (*Clupea harengus*) serve as a model organism in this study. Their foraging behaviour is categorized as a cruising predator (MacKenzie and Kiørboe, 1995), which searches while moving and encounters prey throughout the visual field (Rosenthal and Hempel, 1970). Rosenthal and Hempel (1970) divided swimming patterns in break, slow swimming (meandering/searching), normal swimming and fast swimming (attack) states. Prior to the attack, the larva slows down and performs an S-shape posture. Slow swimming with a large amplitude of the head is assumed to enhance the visual field and the perception time (Rosenthal, 1968). Two developmental stages of larvae were investigated in this study, because herring larvae show a fast improvement of their foraging ability with an increased feeding success from 5% to about 40% in two weeks (Blaxter and Staines, 1971). Hence, larvae at 5 dph are considered to be larvae at first feeding, whereas larvae at 10 dph are more experienced larvae.

To the best of the authors' knowledge, the effect of benthic copepods as prey on the foraging behaviour of pelagic fish larvae remains unexplored. Therefore, the swimming behaviour of herring larvae (*C. harengus*) together with their feeding success was analysed in relation to different food sources (harpacticoid copepods *Tachidius discipes* and *Tisbe* sp., as well as the rotifer *Brachionus plicatilis*) and food supply methods using a 2D-video analysis by addressing following questions: (1) are pelagic fish larvae able to perceive and feed on benthic prey, (2) do they change their swimming behaviour when encountering differently moving prey, and (3) is the sieve supply method improving the larval feeding success?

3.2. Material and methods

The behaviour of herring fish larvae (*Clupea harengus*) in correspondence to different prey was monitored using 2D-video observations.

3.2.1 Cultivation of herring larvae and prey organisms

The harpacticoid copepods *Tachidius discipes* and *Tisbe sp.* were batch cultured as prey in a temperature-controlled room (17°C ± 1°C) in trays (H 14 cm * W 61 cm * D 43 cm) stacked in a rack and fed daily with *Rhodomonas* sp. Filtered (0.2 µm) Baltic Sea water (16 ± 1 psu) was exchanged weekly. The rotifer *Brachionus plicatilis* was cultured

as prey in a conical tank with aeration from the bottom and fed daily with a *Nannochloropsis*-concentrate (BlueBiotech GmbH, Büsum, Germany). Prior to introducing rotifers in the larval tank, they were enriched for 3 h with S.presso (Selco, INVE Aquaculture, Belgium) following the suggested enrichment protocol provided with the product.

The eggs of a total of 15 Baltic herring females (*C. harengus*) were artificially fertilized with the sperm of a total of 9 males on glass plates and subsequently kept in a glass aquarium until larvae hatched at 10°C. The daily water exchange with filtered and UV-treated Baltic Sea water (17 ± 1 PSU) was 60%. Once hatched, the fish larvae were transferred into two 30 L tanks placed in one big squared tank filled with water for better temperature control. A density of 20 fish larvae L^{-1} was adjusted. One tank was fed daily with nauplii and copepodites (stages N4 – C3, size fractionated with a sieve: 120 – 250 µm) of *T. discipes* (copepod-tank) and the other with *B. plicatilis* (rotifer-tank) at a density of 3000 prey items L^{-1}. A floating sieve (11 cm ø, 200 µm pore size) with a *T. discipes*-culture inside was additionally introduced in the copepod-fed tank for the purpose of adaptation. Larvae that were used for behavioural studies starved for 24 h prior to the video recording.

3.2.2 Video recording

To determine the ability of pelagic fish larvae to perceive benthic copepods, the swimming behaviour of 30 fish larvae was analysed without food (control) and with *T. discipes* as prey by 2D-video observations. In order to analyse potential differences in foraging behaviour in dependence of prey movement, four different food conditions were tested: (1) *T. discipes* and (2) *Tisbe* sp. to compare two harpacticoid copepod species; (3) *T. discipes* via a floating sieve to analyse the influence of an indirect supply method; and (4) *B. plicatilis* to compare a common used live feed species with the harpacticoid copepods. To assess the effect of ontogenetic shifts, all food conditions were tested at two different developmental stages of *C. harengus* (first feeding at 5 dph and experienced feeding at 10 dph). The fish larvae had a mean standard length of 7.73 ± 0.13 mm and 8.20 ± 0.47 mm, respectively. A sieve with the same mesh size which has been used in the larval rearing tank was used in this video-experiment. The feeding trial with *B. plicatilis* was conducted with fish larvae of *C. harengus* grown in the rotifer-tank and the other feeding trials with larvae of *C. harengus* from the copepod-tank, because fish larvae tend to stick to the prey they experienced previously (Rosenthal and Hempel, 1970). The video recordings were conducted at 11°C in a dark temperature controlled room. For each feeding treatment 30 fish larvae were transferred into a small glass aquarium (H 20 cm * W 19 cm * D 14 cm) containing 3 L filtered water as used in

the rearing tanks. The behaviour of *C. harengus* larvae without food (control) was recorded prior to introducing *T. discipes* in the aquarium. The prey density was equal to the rearing tank. To enhance the contrast of the larvae and their prey, the aquarium had three black opaque sides. The 2D-video set-up consisted of a single camera (Sony HDR-XR550VE, 12MPixel, 25 fps) orthogonally orientated to the front-side of the aquarium (Fig. 3.1). The light source was a fibre optic light (KL1500 LD, Schott, Germany) positioned above the tank. The light intensity under the water surface was 17.5 µmol m^{-2} s^{-1}. After a de-stress time of 20 min after handling, fish larval swimming behaviour was recorded for 20 min for each treatment.
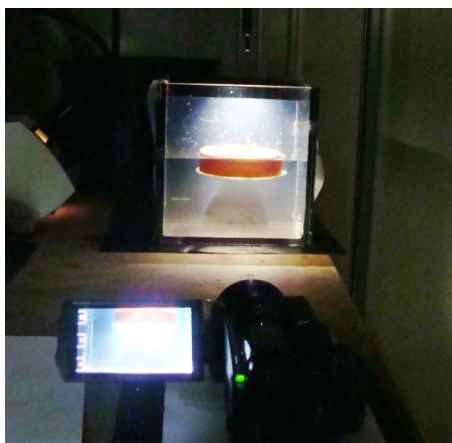

Figure 3.1: Experimental set-up to analyse swimming behaviour of fish larvae. The trial of feeding larvae via a floating sieve is shown.

For each video recording, the trajectories of the fish larvae were reconstructed manually by successively clicking on the position of larval head in each frame, using MATLAB 2009 software (The MathWorks Inc.). Tracks are stored in an array object consisting of x and y coordinates. Only trajectories longer than 7 seconds (175 data points) were used to calculate statistically significant measurements. Each trajectory was considered to be independent. The numerical analysis was applied to an average number of about 4444 data points for each condition.

3.2.3 Analysis of the swimming behaviour

<u>Swimming speed</u>

First the instantaneous speed V_i was calculated for each time step. The distance d (mm) travelled between two successive video frames was computed from the *(x, y)* coordinates as:

$$(1) \qquad d_i = [(x_t - x_{t+1})^2 + (y_t - y_{t+1})^2]^{1/2},$$

where *(x$_t$, y$_t$)* and *(x$_{t+1}$, y$_{t+1}$)* are the positions of fish larva at the time *t* and *t* + 1, respectively.

The swimming speed, V_i (mm s^{-1}) was subsequently estimated as:

(2) $V_i = d_i \cdot f$,

where f is the sampling rate of the camera, i.e. $f = 25$ frames s^{-1}.

Mean swimming speeds were calculated as the average of all instantaneous speeds determined across each individual trajectory.

Swimming states

Based on the swimming speed, swimming states were assigned to each frame. A total of five swimming states were determined according to swimming behaviour described by Rosenthal (1968) and Rosenthal and Hempel (1970) (Table 3.1).

Table 3.1: Swimming states according to larval swimming speed with ecological meaning

Swimming state	Swimming speed (mm s^{-1})	Ecological meaning
Break	< 1, minimal activity to avoid sinking	Aiming for prey (S-shape) or resting
Passive sinking	1 – 5, downward	Resting
Slow swimming	1 – 4	Searching with low energy expenditure
Normal swimming	4 – 50	Searching
Fast swimming	> 50	Attacking

The percentage of total time spent in each swimming state was calculated.

Net-to-gross-displacement-ratio

The net-to-gross-displacement-ratio (NGDR) was used to assess the straightness of the swimming path of larvae of *C. harengus*, according to Buskey (1984).

(3) $NGDR = ND/GD$,

where net displacement (ND) and gross displacement (GD) correspond to the shortest distance from the start to the end point of the trajectory and the actual distance the fish larva had taken, respectively. The ratio is bounded between 0 and 1; a value of 1 reflects a rectilinear movement, whereas a value approaching 0 indicates a large complexity of the swimming path.

Probability density function of angles

The instantaneous angle is defined as the angle between two successive modes and was calculated after Chen et al. (2012). The probability density function (pdf)

describes the relative likelihood for a continuous random variable to have a given value and was calculated to see in more detail the variation of the instantaneous angles.

3.2.4 Feeding larvae

The fed fish larvae of *C. harengus* were subsequently conserved in formalin (4%) to analyse the gut filling. The percentage of larvae which had at least one prey item in the gut (feeding success) was determined for each feeding condition.

3.2.5 Statistical analyses

Prior to statistical analyses, the assumptions of normality and homogeneity of variances were examined. Swimming speed data were log-transformed. The comparison between unfed and *Tachidius*-fed larvae was conducted with a t-test ($\alpha = 0.5$) in the case of swimming speed and NGDR and with a Mann-Whitney-U-test ($\alpha = 0.5$) in the case of swimming states. The effect of both the four different prey types and age of the larvae on the swimming speed was tested by a two factorial ANOVA. Post-hoc comparisons (Tukey unequal N HSD, $\alpha = 0.5$) were performed where appropriate. NGDR and swimming states were analysed with a Kruskal-Wallis-test followed by a multiple comparison test ($\alpha = 0.5$). All statistical tests were conducted with STATISTICA 8. Unless otherwise stated, all values are presented as mean value ± standard deviation.

3.3. Results

3.3.1 Behaviour of unfed larvae versus larvae fed with harpacticoid copepods

The swimming behaviour of unfed larvae (control) and larvae fed with benthic copepods revealed significant differences (Table 3.2). At 5 dph no significant differences (T-test: $t = 1.64$, $p = 0.12$) in mean swimming speed were observed, but the straightness of the path (NGDR) was significantly higher in fed larvae than in unfed larvae (T-test: $t = -2.79$, $p < 0.01$). *Tachidius*-fed larvae spent significant more time in the break state (Mann-Whitney-U test: $U = 6$, $p < 0.01$), but less time in normal swimming ($U = 12$, $p < 0.01$) compared to unfed larvae. Although no significant differences in the time spent in fast swimming were detected ($U = 39$, $p = 0.33$), 6% of the larvae which were fed with *T. discipes* had prey in their gut.

For experienced fish larvae at 10 dph the differences in swimming behaviour between fed and unfed larvae were more pronounced than at first feeding. The mean

swimming speed (T-test: t = -3.10, p < 0.01) and the NGDR of fed larvae (T-test: t = -3.02, p < 0.01) were significantly higher than of unfed larvae, and therefore the swimming path was less tortuous. In addition, fed larvae spent more time in fast swimming than the unfed larvae and less time in the break state (Mann-Whitney-U test: U = 143.5, p < 0.05, both), whereas the time spent in the other states was similar. Both *Tachidius*-fed and unfed larvae did not enter the slow swimming state.

Table 3.2: Analyses of feeding behaviour of 5 and 10 dph herring larvae without food (control) and with food (*Tachidius discipes*). Mean ± SD are displayed, (N = 10 (5 dph), 20 (10 dph))

Larval age (dph)	Feeding condition	Swimming speed (mm s^{-1})	NGDR	feeding larvae (%)	% of time spent in each swimming state ± 95% CI				
					break	sink	slow	normal	fast
5	Control	5.6 ± 2.1	0.10 ± 0.10*	-	13.3 ± 2.3*	14.2 ± 4.3	19.8 ± 4.0	52.2 ± 9.7*	0.5 ± 1.0
	Tachidius	4.4 ± 1.9	0.24 ± 0.12*	6	31.9 ± 9.5*	19.9 ± 3.5	17.6 ± 4.1	30.5 ± 5.9*	0.04 ± 0.06
10	Control	8.4 ± 2.1*	0.44 ± 0.19*	-	9.7 ± 1.8*	12.6 ± 2.9	-	77.6 ± 4.6	0.2 ± 0.2*
	Tachidius	10.6 ± 2.5*	0.61 ± 0.17*	22	7.0 ± 2.4*	9.8 ± 4.6	-	82.0 ± 7.1	1.1 ± 0.8*

NGDR = Net to gross displacement ratio; CI = Confidence interval;
Superscript * within same larval age indicate significant differences

A total of six angles between two movements showed higher probabilities: around 0, 23, 41, 86, 131 (young larvae), 140 (old larvae) and 180° (Fig. 3.2). The 23° and 41° angles were more pronounced under feeding condition than in the control, whereas an angle of 130° or 140° was more pronounced in the control compared to the feeding treatment. Comparing 5 and 10 dph larvae, the higher angles of 130° and 140° have a higher probability in younger larvae, whereas angles of 23 and 41° are more likely in older larvae.
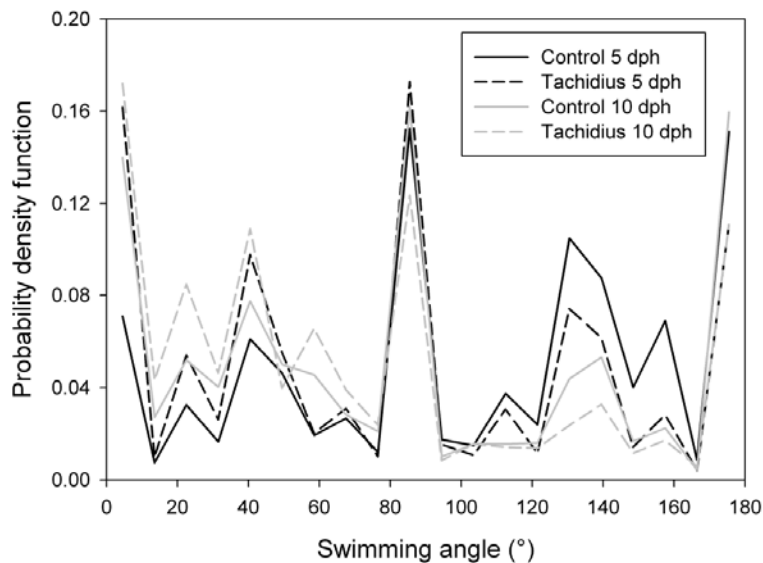

Figure 3.2: Probability density functions of instantaneous swimming angle in unfed herring larvae (control) and larvae fed with *Tachidius discipes* at two different developmental stages (5 and 10 dph).

3.3.2 Behaviour of larvae fed with different prey types

Swimming speed

The mean swimming speed of the herring larvae increased significantly with ontogeny (ANOVA: $F = 202.71$, $p < 0.001$) in fed larvae up to 12 mm s^{-1} (Fig. 3.3). However, fish larvae did not change the mean swimming speed when fed with different prey types.
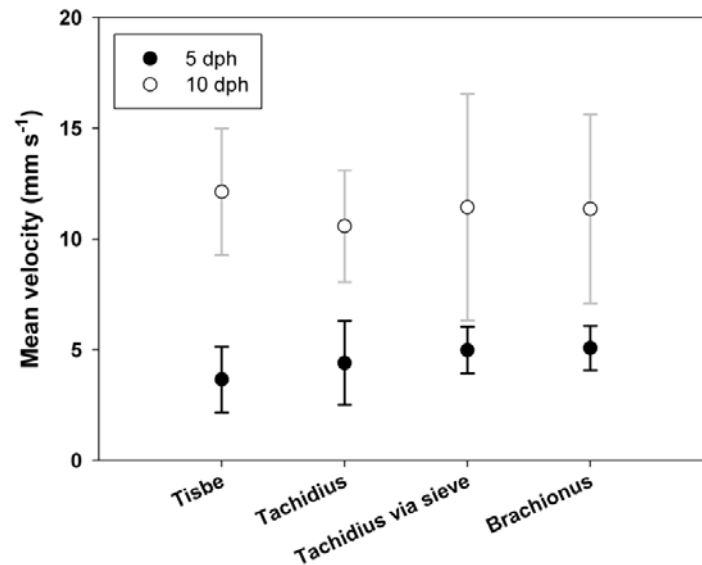

Figure 3.3: Swimming speed of 5 days and 10 days old herring larvae (*Clupea harengus*) in dependence of prey type (*Tachidius discipes*, *Brachionus plicatilis*, *Tisbe* sp.) and supply method (*T. discipes* offered via a floating sieve). The mean and standard deviation are displayed ($N_{Trajectories} = 10-20$).

Swimming states

The swimming behaviour of first feeding larvae (5 dph) did not exhibit a clear dominance for a certain state (Fig. 3.4 A). Larvae which were fed with *Tachidius* via a sieve spent significantly more time in normal swimming than larvae fed directly with *Tachidius* ($p < 0.01$). Furthermore, larvae fed with *Tisbe* spent significantly more time in the break state compared to fish larvae fed with *Tachidius* via a sieve ($p < 0.05$) or with *Brachionus* ($p < 0.01$). The larvae entered seldom the fast swimming state ($0 - 0.6\%$) regardless of food source.

The more experienced fish larvae at 10 dph exhibited two main activity patterns (Fig. 3.4 B). Directly copepod (*Tachidius* and *Tisbe*) fed larvae spent significantly more time in the normal swimming state than *Brachionus* ($p < 0.001$, both) and *Tachidius* via sieve fed larvae ($p < 0.001$ and $p < 0.01$, respectively), but significantly less time in the slow swimming state ($p < 0.05$, all comparisons). Directly *Tachidius*-fed larvae did not enter the slow swimming state during the experimental observation. When *Tachidius* was

provided via a floating sieve, the behaviour resembled the behavioural pattern of *Brachionus*-fed larvae. The time spent in fast swimming did not change significantly between prey types (Kruskal-Wallis: H = 12.97, p = 0.34), but exhibited a range between 1.15% (*Tachidius*-fed larvae) and 2.29% (*Brachionus*-fed larvae).

Comparing the two larval stages (5 dph and 10 dph), the behavioural pattern of younger larvae did not show clear dominance for one state, whereas the pattern of the older larvae was dominated by normal swimming. The time spent in normal swimming increased significantly with ontogeny (p < 0.05, *Brachionus* and *Tachidius* via sieve; p < 0.001, *Tachidius* and *Tisbe*), whereas the time spent in break state decreased significantly (p < 0.001, all observations). Furthermore, fish larvae of all feeding regimes decreased the time spent in sinking (p < 0.01, all observations) with ontogeny, with the exception of larvae feeding on *Tachidius* via a sieve (p = 0.14). The time spent in slow swimming increased significantly in larvae feeding on *Tachidius* via a sieve (p < 0.05) and on *Brachionus* (p < 0.01), but decreased when directly feeding on *Tachidius* (p < 0.001) and *Tisbe* (p < 0.05) with ontogeny. The time spent in fast swimming did not change significantly with ontogeny, but an increasing trend was observed.
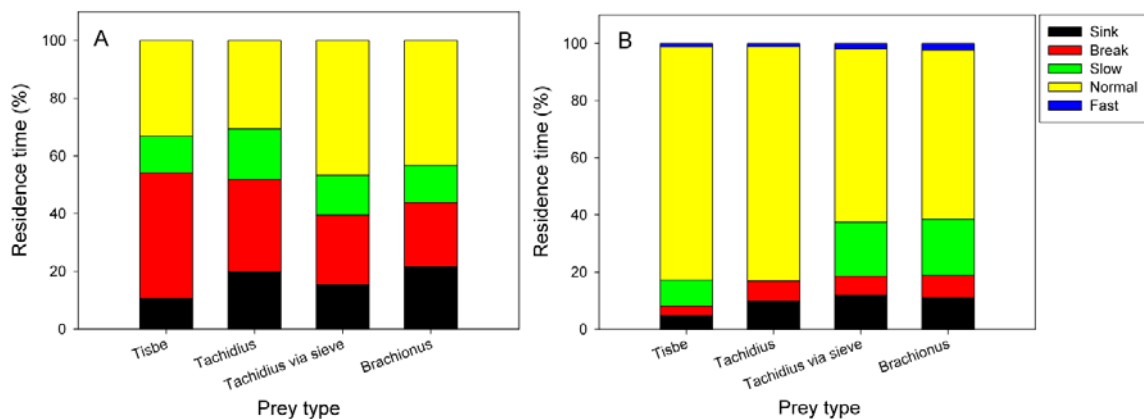

Figure 3.4: Larval swimming behaviour of (A) 5 days old and (B) 10 days old herring larvae (*Clupea harengus*) in dependence of prey type (*Tachidius discipes*, *Brachionus plicatilis*, *Tisbe* sp.) and supply method (*T. discipes* offered via a floating sieve). Mean of residence time (%) spent in each of the five states.

NGDR

The NGDR increased with ontogeny of the larvae (Kruskal-Wallis: H = 48.36, p < 0.01), but the food source had no effect (Kruskal-Wallis: H = 3.62, p = 0.31 (5 dph) and H = 6.33, p = 0.097 (10 dph)) (Fig. 3.5). The higher the ratio, the straighter is the swimming path. Although not significant, the NGDR was highest at *Tachidius*-fed larvae at 10 dph.
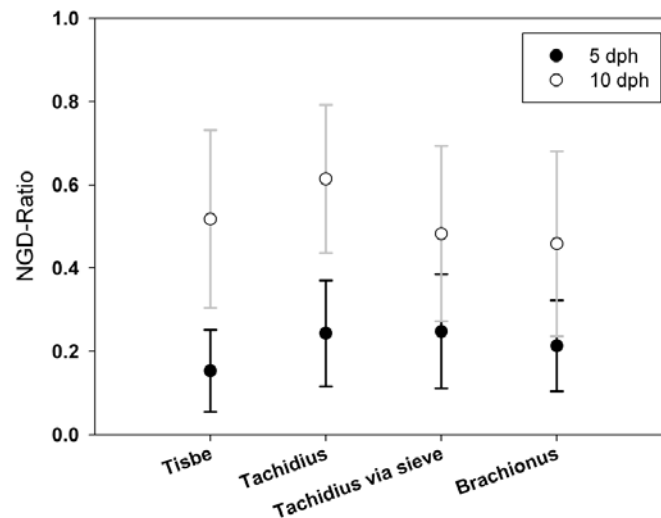
Figure 3.5: Net-to-gross-displacement-ratio (NGDR) of 5 dph and 10 dph old herring larvae (*Clupea harengus*) in dependence of prey type (*Tachidius discipes*, *Brachionus plicatilis*, *Tisbe* sp.) and supply method (*T. discipes* offered via a floating sieve). Mean and standard deviation are displayed ($N_{Trajectories}$ = 10-20).

Probability of instantaneous angles

For each feeding condition, six angles between two movements showed higher probabilities: around 0, 23, 41, 86, 131 (first feeding larvae), 140 (experienced larvae) and 180° (Fig. 3.6 A, B). At 5 dph, the prey type did not affect the swimming angle.

At 10 dph the low angles between 20 and 60° are more pronounced than the high angles between 120 and 160°. The probability distribution of swimming angle of *Tachidius*-fed larvae was similar to larvae fed with *Tachidius* via a sieve, whereas *Brachionus*-fed larvae had a lower probability of low angles and higher probability of high angles compared to the other feeding conditions. *Tisbe*-fed larvae had a low probability to swim in an angle of 180°.
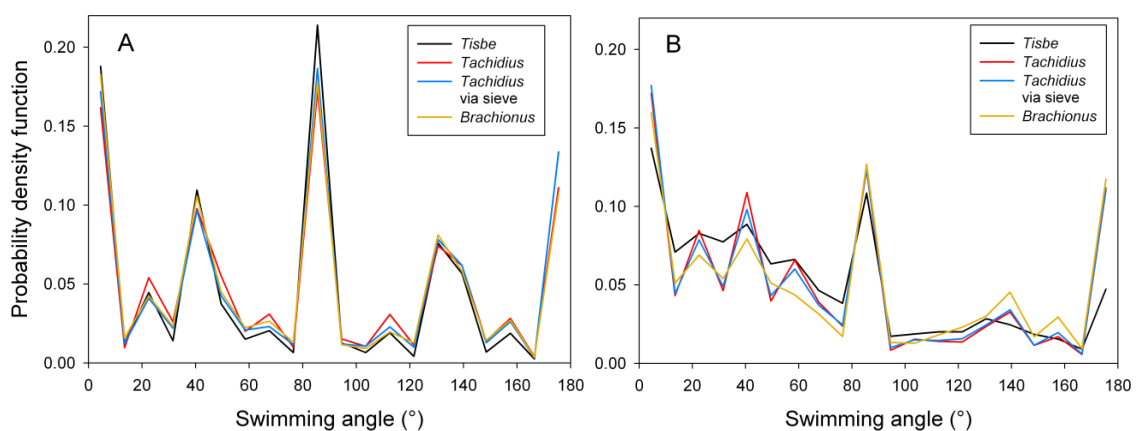

Figure 3.6: Probability density functions of instantaneous angle in dependence of prey type (*Tachidius discipes*, *Brachionus plicatilis*, *Tisbe* sp.) and supply method (*T. discipes* offered via a floating sieve) for (A) 5 dph and (B) 10 dph old herring larvae (*Clupea harengus*).

<u>Feeding larvae</u>

The proportion of larvae which had at least one prey item in their gut differed between food source (Fig. 3.7). When *Brachionus* was the available prey, 46% of the larvae had prey in their gut at 5 dph. This proportion decreased to 29% at 10 dph. When feeding on *Tisbe* the larvae did not show a difference in feeding success (around 30%) with ontogeny. Although increasing with ontogeny, the lowest feeding success showed larvae fed directly with *Tachidius*. Especially at 5 dph, the feeding success of larvae fed via a sieve was 20% higher compared to directly *Tachidius*-fed larvae.
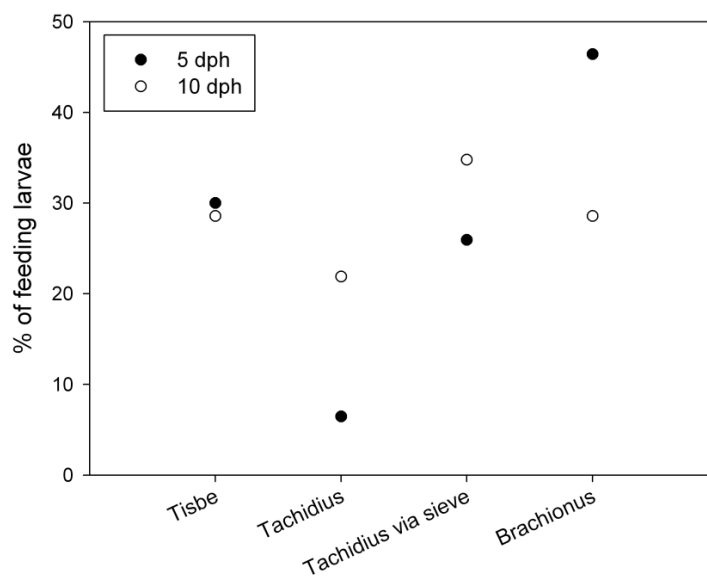

Figure 3.7: Proportion of larvae which had at least one prey item in the gut (feeding success) in dependence of prey type (*Tachidius discipes*, *Brachionus plicatilis*, *Tisbe* sp.) and supply method (*T. discipes* offered via a floating sieve) at two different larval development stages (5 and 10 dph).

3.4. Discussion

Overall, the interpretation of the swimming states, especially swimming and break, depends on the larva's foraging type. Predators with a cruise strategy are searching while moving and rarely pause, whereas predators with a pause-travel or saltatory strategy are exclusively searching while pausing and swim to enter a new unscanned water volume (O'Brien et al., 1990). Results in this study show that first-feeding herring larvae spent around one third in pausing. This is in agreement with observations made by Rosenthal and Hempel (1970), but contrasts with the description for cruising predators. At first feeding, fish larvae are subject to physical constraints imposed by both

viscous and inertial realms, with a Reynolds number of around 38 (based on mean swimming speed: 5 mm s^{-1}). Moreover, the swimming ability is low and herring larvae have a low perception distance of 2 to 8 mm. Since perception distance is higher at resting and slow swimming compared to normal swimming (Rosenthal and Hempel, 1970) and because energy expenditure increases with swimming speed (Dabrowski et al., 1988), early stage fish larvae can benefit from pausing due to lower energy expenditure and a larger visual field at the same time.

More experienced fish larvae (10 dph) have developed a higher swimming ability, which led to a higher mean swimming speed. This in turn results in a shift to a realm where inertial forces start to prevail. This is expressed in a higher Reynolds number of around 88 (based on mean swimming speed: 11 mm s^{-1}) compared to 38 five days earlier. Consequently, the herring larvae benefit from higher swimming activities by a reduced influence of drag forces and an increase of prey encounter rate by increasing the explored water volume.

In summary, the behaviour changes with ontogeny by having a tendency to a pause-travel-predator at first feeding (high proportion of break state) and subsequently changing more to a cruise searcher (dominance of normal swimming). As a consequence, besides normal and slow swimming also pausing is considered as a mode of searching, but with decreasing energy expenditure from normal swimming to pausing. Additionally, the break state can be an indication that a larva was in the S-posture immediately before an attack (Rosenthal, 1969), as this could not be distinguished with the present set-up.

3.4.1 Are pelagic fish larvae able to perceive benthic prey?

In this study, herring larvae showed significant differences between food and no food conditions. The swimming path was straighter at feeding conditions, suggesting a more target-orientated swimming. Furthermore, fed larvae showed a higher probability in the swimming angle of around 41° than the control. This is considered to be an attack angle, since juvenile herring (Thetmeyer and Kils, 1995) as well as larval cod (Hunt von Herbing and Gallager, 2000) attack their prey at this angle.

The observed difference of response between old and young larvae in time of each swimming state is probably due to the general different feeding strategy of young (pause-travel) and older (cruising) larvae. The increased swimming activity in young non-fed larvae is also typical for larvae exposed to a low food density in order to increase their encounter rate with prey (Munk and Kiørboe, 1985). Moreover, the feeding success of 6% and 22% in young and old larvae, respectively, indicates firstly that foraging abilities

improved considerably during 5 days and secondly that herring fish larvae are able to capture benthic copepods. However, this feeding success is a bit lower compared to 40% in *Artemia*-fed larvae at 14 dph reported by Blaxter and Staines (1971).

3.4.2 Do they change their foraging behaviour when encountering differently moving prey?

First feeding fish larvae have limited abilities to adjust their swimming behaviour according to the prey type, since early stage fish larvae have only pectoral fins and a finfold (Doyle, 1977; Hunt von Herbing, 2001). As a consequence, the locomotion ability is reduced at 5 dph and the swimming patterns are more similar when exposed to varying prey types compared to older larvae. However, first feeding larvae spent more time in pausing when feeding on elusive harpacticoid copepods compared to slow moving rotifers. They probably pause more in order to ensure a longer presentation of the faster copepods to the retina and they need presumably more time to adjust their S-shape position prior to attack (Rosenthal and Hempel, 1970).

The general low locomotory capability is also an explanation for the lower feeding success when feeding on more elusive copepods than slow moving *Brachionus*. In general, rotifers have poor escape abilities and they do not increase their speed when larvae were present (Turingan et al., 2005). At predator presence, *Brachionus rotundiformis* exhibits a swimming speed of 1.33 ± 0.12 mm s^{-1} compared to 17.3 ± 1.7 mm s^{-1} of a harpacticoid copepod (*Nitokra lacustris*) (Beck and Turingan, 2007). Other species of fish larvae, such as cod and red drum larvae also preferred slow moving prey at first feeding and switched later on to more elusive prey (Hunt von Herbing and Gallager, 2000; Krebs and Turingan, 2003).

As mentioned above, all larvae switched more to a cruising behaviour at 10 dph. Apparently, directly copepod-fed larvae spent more energy for searching than rotifer-fed larvae, since they spent more time in normal swimming, which consumes more energy than slow swimming (Dabrowski, 1986). A higher swimming activity is typical for larvae encountering low prey densities (Munk and Kiørboe, 1985). Thus, the higher swimming activity of directly copepod-fed larvae suggests that the benthic copepods provided a lower prey density in the water column and remained more at the bottom of the aquarium due to their benthic mode of life. With an increased swimming activity the larvae enhance their encounter rate with prey.

In general, the larval foraging performance is improving with ontogeny (Blaxter and Staines, 1971; Kiørboe et al., 1985), the pigmentation of the eye is enhancing (Chesney, 2007) and the prey perception distance increases with larval size (Miller et al., 1988). This is reflected in the higher feeding success of *Tachidius*-fed larvae at 10 dph than at

5 dph. In contrast, the percentage of rotifer-fed larvae with prey in their gut had unexpectedly decreased at 10 dph. This is also reflected in the lower probability of the attack swimming angle at around 41° (Thetmeyer and Kils, 1995) which was highest in rotifer-fed larvae at 5 dph, but decreased in the older stage. The stimulating effect of the copepods' movement (Heath and Moore, 1997) as well as the chemical stimuli (Dempsey, 1978) probably start to play a bigger role, since herring larvae showed increased activity to glycine and proline (Dempsey, 1978), two amino acids which are more abundant in copepods than in rotifers (van der Meeren et al., 2008).
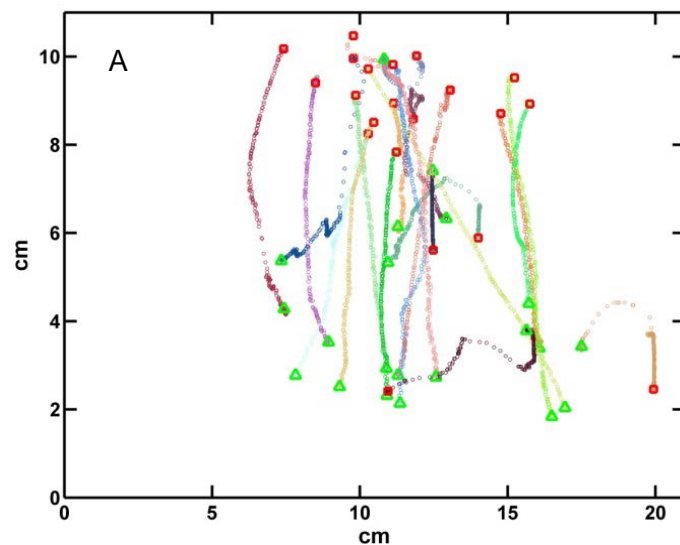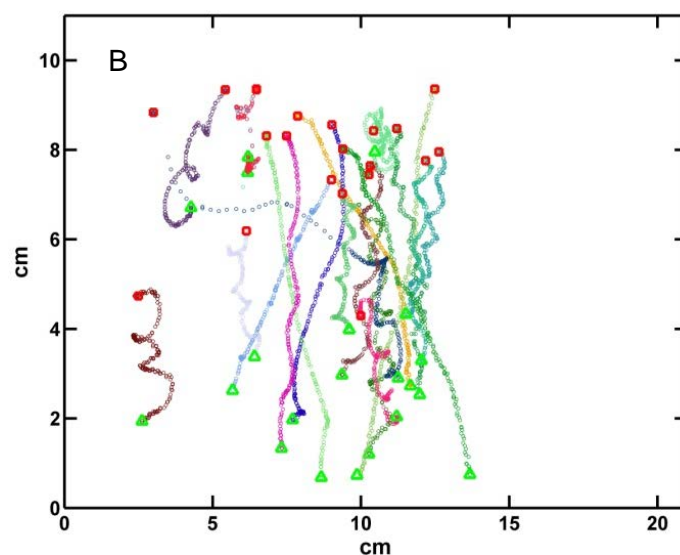

Figure 3.8: Trajectories of herring larvae A) fed directly with *Tachidius discipes* and B) fed directly with *Tisbe* sp. at a larval age of 10 dph.
Δ = start point, o = end point of each trajectory. The higher the distance between the points, the higher the swimming speed of the larva.

The comparison of *Tisbe*- and *Tachidius*-fed larvae revealed that *Tisbe*-fed larvae spent more time in slow-swimming at 10 dph and their feeding success was almost 7% higher than with *Tachidius*. The observation that fish larvae presumably slow down when

encountering food patches in order to stay longer therein (Rosenthal, 1968), suggests that *Tisbe*-fed larvae experienced higher food densities although the same food density was established in all feeding trials. Furthermore, the trajectories in Fig. 3.8 revealed that *Tisbe*-fed larvae swam often in a convoluted way with decelerating and accelerating components compared to *Tachidius*-fed larvae. This behaviour also results in a longer stay in the same water volume. The deceleration of the fish larvae also reduced the predator perception of copepods, which depends on fluid deformation rate caused by the fish larva (Kiørboe and Visser, 1999). This is confirmed by the higher feeding success of *Tisbe*-fed larvae in this study.

Moreover, the two copepod species might have different escape behaviour and/or predator perception abilities in general. For instance, in calanoid copepods, a hop-and-sink swimming pattern revealed shorter response latencies to acoustic signals than a cruising pattern (Waggett and Buskey, 2007). Behavioural analyses of benthic harpacticoid copepods are scarce (Hwang and Turner, 1995) and were not part of this study either. However, adult stages of *Tachidius* were positively phototactic, whereas adult stages of *Tisbe* avoided high light intensities and were harder to capture with a pipette (own observation). Therefore, a higher feeding success with *T. discipes* would have been expected. However, the capture attempts with the pipette were done from above the copepod, whereas fish attack from below the prey (Thetmeyer and Kils, 1995), consequently this observation might be misleading. Differences in predator perception abilities of the two copepod species were likely the reason for the different feeding success of the fish larvae (Titelman, 2001; Titelman and Kiørboe, 2003). In conclusion, the results tend to show that *Tisbe* is a superior food source for pelagic marine fish larvae compared to *Tachidius*.

3.4.3 Is the sieve supply method improving the larval feeding success?

The swimming behaviour of copepod via sieve-fed larvae resembled the swimming behaviour of rotifer-fed larvae at 10 dph, whereas directly copepod-fed larvae were more active and spent less time in slow swimming (Fig. 3.4 B). Since metabolic costs increase with swimming speed (Hunt von Herbing et al., 2001), the provision of copepods via a sieve or feeding the larvae with rotifers reduced the energy demand and resulted probably in a higher net energy gain, assuming that both prey types provide similar energetic value.

Although the probability distribution of swimming angles is similar in both supply methods, including the attack angle (Fig. 3.6 A+B), the feeding success is higher in copepod via sieve-fed larvae. Copepods that fall through the sieve often do not move

and sink passively to the bottom until they start active swimming (own observation). Thus, slowly sinking copepods might be easier to capture than active swimming copepods.

In conclusion, pelagic fish larvae are able to attack and feed on benthic copepods, although the energy demand seems to be higher when the copepods were introduced directly in the fish tank. On the contrary, the foraging behaviour on copepods offered via a sieve resembled the behaviour when feeding on rotifers and the feeding success was higher compared to directly fed copepods. The provision of harpacticoid copepods via a sieve combines the advantageous characteristic of rotifer-fed larvae, i.e. high capture success and presumably low energy expenditure but also the high nutritional value of copepods, which has been shown in numerous studies (McEvoy et al., 1998; Rønnestad et al., 1998; Payne et al., 2001). Consequently, when harpacticoid copepods are used as food organisms, their provision via a floating sieve is recommended to improve the rearing of marine fish larvae. Additionally *Tisbe* should be preferred over *Tachidius* as harpacticoid species, since feeding success was higher with the first species. Nevertheless, at very first feeding (5 dph) slow moving prey such as rotifers (this study) or protozoa (Hunt von Herbing and Gallager, 2000) should be preferred.

Chapter 3

Discussion

Discussion

Discussion

In this study the suitability of harpacticoid copepods as food for marine fish larvae was investigated with the focus on fatty acids as a determinant of nutritional value. Three harpacticoid copepod species originating from the Kiel Fjord were identified as *Ameira parvula*, *Amonardia normani* and *Tachidius discipes*. *A. parvula* showed a low reproduction and the nauplii stages of *A. normani* were not able to swim which makes them unavailable for pelagic fish larvae. Consequently, the first two species are not considered to be suitable food items, but *T. discipes* was chosen for further experiments.

Suitability of *Tachidius discipes*

The first chapter revealed that *T. discipes*, a quite abundant harpacticoid copepod in the Baltic Sea, fulfils almost all criteria summarized by Uhlig (1984) for potential candidates in aquaculture.

This species has a relatively short life cycle with a duration of ca. 11 days from nauplius to adult stage, which is similar to other harpacticoid species such as *Tigriopus japonicus* (Provasoli et al., 1959), *Euterpina acutifrons* (Zurlini et al., 1978) and *Tisbe* sp. in this study.

Furthermore, *T. discipes* has a reproductive capacity comparable to other harpacticoid copepods (Zurlini et al., 1978). Including the feasible copepod density in the calculation, the production of *T. discipes* nauplii can be 8-times higher than the production of calanoid copepods (*Acartia tonsa*, (Peck and Holste, 2006)).

The mean copepod density (all stages) in mass culture was 91 individuals cm^{-2} (15 000 individuals L^{-1}), thus 5 times higher compared to calanoid cultures (Chesney, 1989), revealing a tolerance of high culture densities. Zhang and Uhlig (1993) proposed an even higher density of 40 harpacticoid females cm^{-2} (= 10 000 females L^{-1}) for maximum productivity. Since harpacticoids require surface area rather than water volume, increasing the surface normally improves the productivity. However, Støttrup and Norsker (1997) could not achieve a higher harvest yield with their half-automatic cultivation system in which they used plastic balls to increase the surface area in conical tanks. Individuals probably cling to the substrate, which hampers the harvesting and leads to lower yields.

The tolerance of *T. discipes* to salinity or temperature changes was not investigated in this study, but their origin from the eulittoral suggests a general tolerance

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of physical changes. Smol and Heip (1974) additionally observed high survival rates at temperatures between 5 and 15°C.

However, the acceptance of diverse food sources was not fulfilled by *T. discipes* to the same degree as by *Tisbe* sp. Alternative food sources such as vegetables and enriched tomato juice, which were successfully used as food for *Tisbe* sp. (Kahan, 1979) and *Nitokra lacustris* (Rhodes, 2003), resulted in a low performance of both species (*T. discipes* and *Tisbe* sp.) in this study (unpublished observation by the author). However, *Tisbe* sp. performed better when fed with algae of low PUFA content. It either has a higher fatty acid desaturation capacity or uses bacteria when exposed to food sources that are low in highly unsaturated fatty acids. The description of *Tisbe* spp. as an opportunistic species (Castel and Lasserre, 1979; Gee et al., 1985; Gollasch et al., 2000) indicates its flexibility. The ability of using bacteria as a food source can have advantages by compensating inadequate food. On the other hand, this can also have disadvantages when trying to influence the nutritional value of *Tisbe* sp. by providing a certain microalgal species, but mainly bacteria are ingested instead. Nevertheless, when *T. discipes* is fed with *Rhodomonas* sp. or *Phaeodactylum tricornutum* it shows similar performance to *Tisbe* sp. Furthermore, it meets the DHA and EPA-content required for fish larvae, such as *Psetta maxima* and *Sparus aurata* (reviewed by Izquierdo and Koven, 2011), as well as the ratio of DHA:EPA recommended by Sargent et al. (1999a). In summary, *T. discipes* is a highly reproductive species which is easy to culture in high densities. It requires only small volumes and provides a high nutritional value in terms of fatty acids.

However, the low performance of herring larvae fed with *T. discipes* (Chapter 2) emphasised the importance to define some additional criteria. The subsequent digestibility test revealed interspecific differences of harpacticoid copepods in protein digestibility using trypsin, the major pancreatic enzyme in first feeding fish larvae (Pedersen and Andersen, 1992). *T. discipes* was less digestible than *Tisbe* sp. Consequently, robustness, which was higher in *T. discipes* in terms of handling-stress, is obviously increased at the expense of digestibility.

Both observed differences between *T. discipes* and *Tisbe* sp., usage of other food sources and digestibility, result probably from their adaptation to different habitats. In this study, *T. discipes* originated from a coastal zone with coarse sand and had to withstand high ranges of environmental changes, especially mechanical disturbance, which probably led to a stiffened cuticle with closely linked segments. On the contrary, the natural habitat of *Tisbe* sp. is often the phytal (Hicks, 1980). This living mode implies the usage of bacteria since copepods do not graze directly on macroalgae, but on populations of bacteria, diatoms, fungi and blue-green algae, which are associated to the

mucilage released by macroalgae (Hicks and Coull, 1983). Therefore, *Tisbe* sp. is probably more used to a bacterial based diet and developed a higher desaturation capacity.

In conclusion, it is good to have a robust species to facilitate the rearing procedure and to reduce the probability of sudden culture break-downs, but robustness may be connected with a lack of digestibility. Thus, future copepod screening should be done in habitats where copepods are exposed to

- low food quality, which enhances their fatty acid desaturation capacity,
- changes in temperature and salinity, which increases their tolerance range and in turn facilitates the rearing,
- low mechanical disturbance, which increases their digestibility.

Moreover, the swimming ability of the new species and its effect on energy expenditure and capture success of fish larvae should be known, as this is decisive for a positive net energy gain of fish larvae (Hunt von Herbing et al., 2001). Only a few studies deal with the swimming behaviour of harpacticoids (Hwang and Turner, 1995; Seifried and Dürbaum, 2000; Turingan et al., 2005). Thus, the knowledge about escape behaviour and predator perceiving abilities of harpacticoids is scarce. However, as demonstrated in chapter 3, these characteristics influence the capture success of fish larvae and their consequential net energy gain, particularly at first feeding when foraging abilities are still low. Therefore behavioural studies of harpacticoid copepods are strongly recommended.

Furthermore, this study revealed that fish larvae adjust their feeding behaviour in response to the swimming behaviour of their prey (Chapter 3). However, this is less pronounced in first feeding than in more experienced fish larvae. Hence, a slow moving prey is recommended at first feeding, due to the low swimming abilities of the fish larvae. This compares well with observations made for first-feeding cod larvae which preferred slowly swimming protozoa (Hunt von Herbing and Gallager, 2000).

In conclusion, when harpacticoid species or live feed species in general are evaluated as food for marine fish larvae, it is recommended to determine their digestibility as well as their swimming behaviour and the associated energetic expenditure of fish larvae.

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General suitability of copepods

The nutritional suitability of copepods has been proven in numerous studies (McEvoy et al., 1998; Payne et al., 2001; Evjemo et al., 2003). However, calanoid copepods are cultured in relatively low densities and require high value microalgae as food source. Harpacticoid copepods can be reared in relatively high densities but the separation of exuviae and detritus from the animals can be an obstacle for a successful harvest (own observation). However, this problem can be circumvented or at least reduced by culturing the copepods directly in the fish tank in a floating sieve (Kahan et al., 1982). Although Zhang and Uhlig (1993) documented with this technique a sufficient provision of 10 nauplii mL^{-1} in the fish larval tank, it has not been tested in a feeding experiment with fish larvae yet. Different temperature optima between copepod and fish larvae might pose a problem and can result in lower copepod productivity. Nevertheless, the provision of harpacticoid copepods via a floating sieve obviously improved the capture success and probably also the net energy gain of herring larvae by spending less time in normal but more time in slow swimming mode. Furthermore, a higher feeding success was demonstrated compared to directly introduced harpacticoid copepods. Hence, the general provision of harpacticoid copepod via a sieve is advantageous, but the combined production of larvae and copepods has to be tested still.

Schipp (2006) stated that it is unlikely that copepods can cost effectively compete with larviculture systems for fish species that are easily cultured on rotifers. The generally high costs of live feeds including rotifers, sudden break downs of live feed production and the potential to introduce pathogenic bacteria or viruses into the fish larval tank led to increased research in formulated feed. This artificial feed is advantageous due to a lower work load, the ability to buy them on demand and its ability to be stored (Barrows and Rust, 2000). However, so far the performance of fish larvae fed with artificial feed is reduced compared to larvae fed with live feed (Blair et al., 2003). This may be due to a low digestibility of the formulated feed as well as a fast leaching of some water-soluble compounds.

Kolkovski et al. (1997) was able to improve the utilization of artificial feed with a supplementation of *Artemia*. The copepods' movement or their chemical stimuli (Dempsey, 1978) even enhanced the feeding incidence in herring larvae older than 9 days after hatching (Chapter 2 and 3) compared to rotifers. Additionally, Støttrup and Norsker (1997) observed an appetite stimulatory effect of *Tisbe holothuriae* when being supplemented for just one day to rotifers as food for turbot larvae. This suggests that copepods are probably a superior supplement to trigger the feeding response in larvae fed with formulated food than other live feed such as rotifers or *Artemia*.

Moreover, harpacticoid copepods have a tank cleaning function (Fig. 1), which can reduce the bacterial load and consequently improve the water quality.
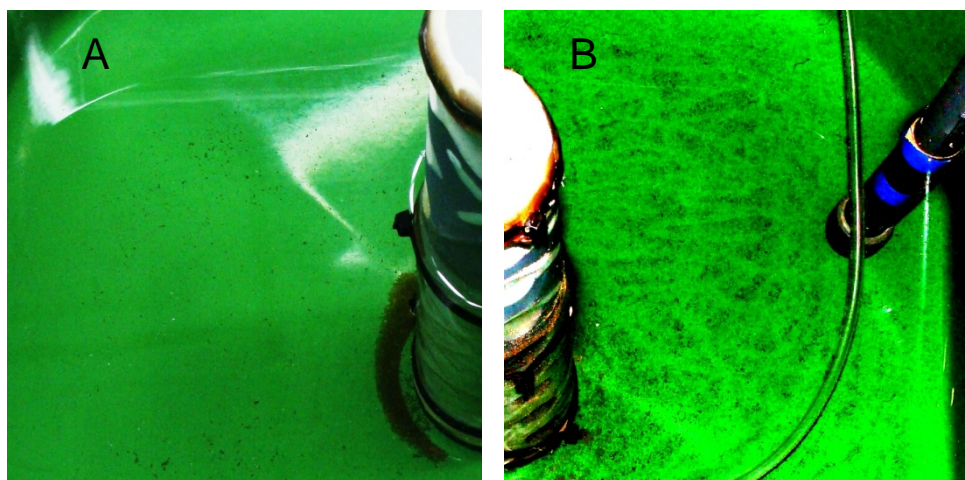

Figure 1: Bottom of a fish larval tank A) with and B) without harpacticoid copepods

In conclusion copepods can be used as a general supplementation to improve the nutritional value of other live or formulated feed and enhance the feeding activity of the fish larvae. Further, they can be used for short-time feeding to ensure optimal pigmentation in larvae of flat fish species, e.g. 14 days after first feeding is a critical time for normal pigmentation in halibut larvae (Rønnestad et al., 1998).

Thus, without the need of a high number of individuals, harpacticoid copepods, which have been selected from the suggested habitat mentioned above, can be easily cultured in batch cultures and provided via a floating sieve to fish larvae and in this way enhance the rearing of marine fish larvae. Moreover, copepods will always be an essential start-feed for new fish species with unknown nutritional requirements and for tropical fish species with small larval sizes.

Outlook

In terms of dietary needs, copepods embody a model for good food quality, since copepods are dietary components of fish larvae in nature. Consequently, they can serve as a reference when designing microdiets or enrichments. Since main nutrients, such as proteins and lipids, are already integrated in enrichment products, the focus should be set on micronutrients. Some of the micronutrients are more abundant in copepods than in rotifers or *Artemia* (Hamre et al., 2008b; van der Meeren et al., 2008) and can be the reason for the superior quality of copepods. Recently, the effect of iodine and selenium enrichments on fish larval performance was investigated with consistently positive results

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(Hamre et al., 2008a; Penglase et al., 2010; Ribeiro et al., 2011). In all studies with enrichments or formulated feed, the bioavailability of the added substances has to be taken into account, as has been shown e.g. for selenium (Wang and Lovell, 1997) and for the source of fatty acids (Coutteau et al., 1997; Tocher et al., 2008). Further research is needed in this area with an emphasis on determining the required amount of the tested substance.

Apart from the risk of delivering pathogenic bacteria, beneficial bacteria such as *Bacillus* spp. and *Roseobacter* sp. are associated with copepods (Hansen and Bech, 1996; De Troch et al., 2010) and can support the health of the fish larva (Gatesoupe, 1999; Planas et al., 2006). Since copepods might provide different bacteria than rotifers, the bacterial colonization of the digestive tract of fish larvae would be influenced by the food source. Additionally, the bacteria of the live feed can be influenced by providing probiotic bacteria which ideally reduce pathogenic bacteria by competitive exclusion (Villamil et al., 2003). The provision of probiotics can also improve the performance of copepods (Drillet et al., 2011), which in turn leads to a higher harvesting yield. Furthermore, the apparent usage of bacteria as food source observed in this study and the observation that harpacticoid copepods increase their faecal pellet production to create a substrate for bacteria (De Troch et al., 2009), which potentially serve as an upgrade of the initial food source (De Troch et al., 2010), emphasises the importance of bacteria in general. Consequently, investigations about the role of bacteria should be intensified.

References

Alheit, J., Scheibel, W., 1982. Benthic harpacticoids as a food source for fish. Marine Biology. 70, 141-147.

Anderson, M., Gorley, R., Clarke, K., 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. Primer-E, Plymouth, UK.

Arthur, D.K., 1976. Food and feeding of larvae of three fishes occurring in the California Current, *Sardinops sagax*, *Engraulis mordax*, and *Trachurus symmetricus*. Fishery Bulletin. 74, 517-530.

Båmstedt, U., 1986. Chemical composition and energy content. in: Corner, E.D.S., O'Hara, S.C.M. (Eds.), The biological chemistry of marine copepods. Clarendon Press, Oxford, pp. 1-58.

Barrows, F.T., Rust, M.B., 2000. Larval feeding–fish. in: Stickney, R.R. (Ed.), Encyclopedia of aquaculture. John Wiley & Sons, Inc., New York, pp. 465-469.

Beck, J., Turingan, R., 2007. The effects of zooplankton swimming behavior on prey-capture kinematics of red drum larvae, *Sciaenops ocellatus*. Marine Biology. 151, 1463-1470.

Bell, M.V., Tocher, D.R., 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. in: Arts, M.T., Brett, M.T., Kainz, M. (Eds.), Lipids in aquatic ecosystems. Springer, New York, pp. 211-236.

Bender, K., Kleinow, W., 1988. Chemical properties of the lorica and related parts from the integument of *Brachionus plicatilis*. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 89, 483-487.

Benítez-Santana, T., Masuda, R., Juárez Carrillo, E., Ganuza, E., Valencia, A., Hernández-Cruz, C.M., Izquierdo, M.S., 2007. Dietary n-3 HUFA deficiency induces a reduced visual response in gilthead seabream *Sparus aurata* larvae. Aquaculture. 264, 408-417.

Berggreen, U., Hansen, B., Kiørboe, T., 1988. Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: Implications for determination of copepod production. Marine Biology. 99, 341-352.

Bergmans, M., 1981. A demographic study of the life cycle of *Tisbe furcata* (Baird, 1837) (Copepoda: Harpacticoida). Journal of the Marine Biological Association of the United Kingdom. 61, 691-705.

Betouhim-El, T., Kahan, D., 1972. *Tisbe pori* n. sp. (Copepoda: Harpacticoida) from the Mediterranean coast of Israel and its cultivation in the laboratory. Marine Biology. 16, 201-209.

Blair, T., Castell, J., Neil, S., D'Abramo, L., Cahu, C., Harmon, P., Ogunmoye, K., 2003. Evaluation of microdiets versus live feeds on growth, survival and fatty acid composition of larval haddock (*Melanogrammus aeglefinus*). Aquaculture. 225, 451-461.

Blaxter, J., Staines, M.E., 1971. Food searching potential in marine fish larvae. in: Crisp, D.J. (Ed.), Fourth European Marine Biology Symposium. University Press, Cambridge, pp. 467-485.

Blaxter, J.H.S., 1965. The feeding of herring larvae and their ecology in relation to feeding. California Cooperative Oceanic Fisheries Investigations Reports. 10, 79-88.

Boxshall, G.A., 1991. Copepoda. in: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Volume 9. Crustacea. Wiley-Liss Inc., New York, pp. 347-384.

Bresciani, J., 1986. The Fine Structure of the Integument of Free-Living and Parasitic Copepods. A Review. Acta Zoologica. 67, 125-145.

Browman, H.I., O'Brien, W.J., 1992a. Foraging and prey search behaviour of golden shiner (*Notemigonus crysoleucas*) larvae. Canadian Journal of Fisheries and Aquatic Sciences. 49, 813-819.

Browman, H.I., O'Brien, W.J., 1992b. The ontogeny of search behavior in the white crappie, *Pomoxis annularis*. Environmental Biology of Fishes. 34, 181-195.

References

Buchet, V., Zambonino Infante, J.L., Cahu, C.L., 2000. Effect of lipid level in a compound diet on the development of red drum (*Sciaenops ocellatus*) larvae. Aquaculture. 184, 339-347.

Buckley, L.J., 1980. Changes in ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and effect of starvation. Fishery Bulletin. 77, 703-708.

Busch, K.E.T., Falk-Petersen, I.-B., Peruzzi, S., Rist, N.A., Hamre, K., 2010. Natural zooplankton as larval feed in intensive rearing systems for juvenile production of Atlantic cod (*Gadus morhua* L.). Aquaculture Research. 41, 1727-1740.

Buskey, E.J., 1984. Swimming pattern as an indicator of the roles of copepod sensory systems in the recognition of food. Marine Biology. 79, 165-175.

Buskey, E.J., 1994. Factors affecting feeding selectivity of visual predators on the copepod *Acartia tonsa*: locomotion, visibility and escape responses. Hydrobiologia. 292, 447-453.

Buskey, E.J., Coulter, C., Strom, S., 1993. Locomotory patterns of microzooplankton: potential effects on food selectivity of larval fish. Bulletin of Marine Science. 53, 29-43.

Buskey, E.J., Lenz, P.H., Hartline, D.K., 2002. Escape behavior of planktonic copepods in response to hydrodynamic disturbances: high speed video analysis. Marine Ecology Progress Series. 235, 135-146.

Cahu, C.L., Zambonino Infante, J.L., 1995. Effect of the molecular form of dietary nitrogen supply in sea bass larvae: response of pancreatic enzymes and intestinal peptidases. Fish Physiology and Biochemistry. 14, 209-214.

Caramujo, M.-J., Boschker, H.T.S., Admiraal, W., 2008. Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. Freshwater Biology. 53, 77-90.

Castel, J., Lasserre, P., 1979. Opportunistic copepods in temperate lagoons of Arcachon Bay: Differential distribution and temporal heterogeneity. Estuarine and Coastal Marine Science. 9, 357-368.

Chandra, R., Liddle, R.A., 2009. Neural and hormonal regulation of pancreatic secretion. Current opinion in gastroenterology. 25, 441-446.

Chen, M.-R., Moison, M., Molinero, J.C., Hwang, J.-S., 2012. Assessing the effect of food and light on *Calanus sinicus* swimming behavior through video-recording experiments. Journal of Experimental Marine Biology and Ecology. 422–423, 14-19.

Chesney, E.J., 1989. Estimating the food requirements of striped bass larvae *Morone saxatilis*: Effects of light, turbidity and turbulence. Marine Ecology Progress Series. 53, 191-200.

Chesney, E.J., 2007. Copepods as live prey: A review of factors that influence the feeding success of marine fish larvae. in: Lee, C.-S., O'Bryen, P.J., Marcus, N.H. (Eds.), Copepods in Aquaculture. Blackwell Publishing Professional, Ames, USA, pp. 133-150.

Christie, W.W., 1989. Gas chromatography and lipids. The Oily Press, Bridgwater.

Ciros-Pérez, J., Gómez, A., Serra, M., 2001. On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. Journal of Plankton Research. 23, 1311-1328.

Clément, P., Wurdak, E., 1991. Rotifera. in: Harrison, F.W., Ruppert, E.E. (Eds.), Microscopic anatomy of invertebrates: Volume 4. Aschelminthes. Wiley-Liss Inc., New York, pp. 219-297.

Clemmesen, C., 1994. The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. Marine Biology. 118, 377-382.

Clemmesen, C.M., 1987. Laboratory studies on RNA/DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. Journal du Conseil International pour l'Exploration de la Mer. 43, 122-128.

Conway, D.V.P., Tranter, P.R.G., Coombs, S.H., 1993. Digestion of natural food by larval and post-larval turbot *Scophthalmus maximus*. Marine Ecology Progress Series. 100, 221-231.

Coutteau, P., Sorgeloos, P., 1997. Manipulation of dietary lipids, fatty acids and vitamins in zooplankton cultures. Freshwater Biology. 38, 501-512.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P., 1997. Review on the dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture. 155, 149-164.

Dabrowski, K., 1986. Energy utilization during swimming and cost of locomotion in larval and juvenile fish. Journal of Applied Ichthyology. 2, 110-117.

Dabrowski, K., Glogowski, J., 1977. Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. Hydrobiologia. 54, 129-134.

Dabrowski, K., Takashima, F., Law, Y.K., 1988. Bioenergetic model of planktivorous fish feeding, growth and metabolism: theoretical optimum swimming speed of fish larvae. Journal of Fish Biology. 32, 443-458.

Dahms, H.-U., Harder, T., Qian, P.-Y., 2007. Selective attraction and reproductive performance of a harpacticoid copepod in a response to biofilms. Journal of Experimental Marine Biology and Ecology. 341, 228-238.

De Troch, M., Cnudde, C., Vyverman, W., Vanreusel, A., 2009. Increased production of faecal pellets by the benthic harpacticoid *Paramphiascella fulvofasciata*: importance of the food source. Marine Biology. 156, 469-477.

De Troch, M., Cnudde, C., Willems, A., Moens, T., Vanreusel, A., 2010. Bacterial colonization on fecal pellets of harpacticoid copepods and on their diatom food. Microbial Ecology. 60, 581-591.

De Troch, M., Boeckx, P., Cnudde, C., Van Gansbeke, D., Vanreusel, A., Vincx, M., Caramujo, M.J., 2012. Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis. Marine Ecology Progress Series. 465, 53-67.

Decho, A.W., Moriarty, D.J.W., 1990. Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. Limnology and Oceanography. 37, 1039-1049.

Dempsey, C.H., 1978. Chemical stimuli as a factor in feeding and intraspecific behaviour of herring larvae. Journal of the Marine Biological Association of the United Kingdom. 58, 739-747.

Desvilettes, C.H., Bourdier, G., Amblard, C.H., Barth, B., 1997. Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. Freshwater Biology. 38, 629-637.

Dhert, P., Rombaut, G., Suantika, G., Sorgeloos, P., 2001. Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture. 200, 129-146.

Dhont, J., Van Stappen, G., 2003. Biology, tank production and nutritional value of *Artemia*. in: Støttrup, J.G., McEvoy, L.A. (Eds.), Live feeds in marine aquaculture. Blackwell Science, Oxford, pp. 65-121.

Doi, M., Toledo, J.D., Golez, M.S.N., de los Santos, M., Ohno, A., 1997. Preliminary investigation of feeding performance of larvae of early red-spotted grouper, *Epinephelus coioides*, reared with mixed zooplankton. Hydrobiologia. 358, 259-263.

Døving, K.B., Mårstøl, M., Andersen, J.R., Knutsen, J.A., 1994. Experimental evidence of chemokinesis in newly hatched cod larvae (*Gadus morhua* L.). Marine Biology. 120, 351-358.

Doyle, M., 1977. A morphological staging system for the larval development of the herring, *Clupea harengus* L. Journal of the Marine Biological Association of the United Kingdom. 57, 859-867.

Drillet, G., Rabarimanantsoa, T., Frouël, S., Lamson, J., Christensen, A.M., Kim-Tiam, S., Hansen, B.W., 2011. Do inactivated microbial preparations improve life history traits of the copepod *Acartia tonsa*? Marine Biotechnology. 13, 831-836.

Eggers, D.M., 1977. The nature of prey selection by planktivorous fish. Ecology. 58, 46-59.

Evjemo, J.O., Reitan, K.I., Olsen, Y., 2003. Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. Aquaculture. 227, 191-210.

FAO, 1997. Technical guidelines for responsible fisheries, Rome, pp. 40.

FAO, 2004. The State of World Fisheries and Aquaculture, Rome, pp. 153.

References

FAO, 2012. The State of World Fisheries and Aquaculture, Rome, pp. 209.

Fernández-Reiriz, M.J., Labarta, U., Ferreiro, M.J., 1993. Effects of commercial enrichment diets on the nutritional value of the rotifer (*Brachionus plicatilis*). Aquaculture. 112, 195-206.

Fernández, I., Hontoria, F., Ortiz-Delgado, J.B., Kotzamanis, Y., Estévez, A., Zambonino-Infante, J.L., Gisbert, E., 2008. Larval performance and skeletal deformities in farmed gilthead sea bream (*Sparus aurata*) fed with graded levels of Vitamin A enriched rotifers (*Brachionus plicatilis*). Aquaculture. 283, 102-115.

Fields, D.M., Yen, J., 1997. The escape behavior of marine copepods in response to a quantifiable fluid mechanical disturbance. Journal of Plankton Research. 19, 1289-1304.

Fleeger, J.W., 2005. The potential to mass-culture harpacticoid copepods for use as food for larval fish. in: Lee, C.-S., O'Bryen, P.J., Marcus, N.H. (Eds.), Copepods in Aquaculture. Blackwell Publishing, Ames, pp. 11-24.

Fossum, P., 1983. Digestion rate of food particles in the gut of larval herring. Fiskeridir. Skr. Ser. Havunders, 347-357.

França, R.C.P., Amaral, I.P.G., Santana, W.M., Souza-Santos, L.P., Carvalho Jr, L.B., Bezerra, R.S., 2010. Proteases from the harpacticoid copepod *Tisbe biminiensis*: Comparative study with enzymes from farmed aquatic animals. Journal of Crustacean Biology. 30, 122-128.

Freeman, J.A., 1989. Segment morphogenesis in *Artemia* larvae. in: Warner, A.H., MacRae, T.H., Bagshaw, J.C. (Eds.), Cell and Molecular Biology of Artemia Development. Plenum Press, New York, pp. 59-76.

Frolov, A.V., Pankov, S.L., Geradze, K.N., Pankova, S.A., Spektorova, L.V., 1991. Influence of the biochemical composition of food on the biochemical composition of the rotifer *Brachionus plicatilis*. Aquaculture. 97, 181-202.

Frost, B.W., 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnology and Oceanography. 17, 805-815.

Fuge, R., 2007. Iodine deficiency: An ancient problem in a modern world. AMBIO: A Journal of the Human Environment. 36, 70-72.

Galbraith, P.S., Browman, H.I., Racca, R.G., Skiftesvik, A.B., Saint-Pierre, J.F., 2004. Effect of turbulence on the energetics of foraging in Atlantic cod *Gadus morhua* larvae. Marine Ecology Progress Series. 281, 241-257.

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture. 180, 147-165.

Gee, J.M., Warwick, R.M., Schaanning, M., Berge, J.A., Ambrose, W.G., 1985. Effects of organic enrichment on meiofaunal abundance and community structure in sublittoral soft sediments. Journal of Experimental Marine Biology and Ecology. 91, 247-262.

Gollasch, S., Lenz, J., Dammer, M., Andres, H.G., 2000. Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. Journal of Plankton Research. 22, 923-937.

Guérin, J.P., Rieper-Kirchner, M., 1991. Influence of three bacteria strains on the population dynamics of *Tisbe holothuriae* (Copepoda, Harpacticoida). Helgoland Marine Research. 45, 493-511.

Hamre, K., Mollan, T.A., Sæle, Ø., Erstad, B., 2008a. Rotifers enriched with iodine and selenium increase survival in Atlantic cod (*Gadus morhua*) larvae. Aquaculture. 284, 190-195.

Hamre, K., Srivastava, A., Rønnestad, I., Mangor-Jensen, A., Stoss, J., 2008b. Several micronutrients in the rotifer *Brachionus* sp. may not fulfil the nutritional requirements of marine fish larvae. Aquaculture Nutrition. 14, 51-60.

Hansen, B., Bech, G., 1996. Bacteria associated with a marine planktonic copepod in culture. I. Bacterial genera in seawater, body surface, intestines and fecal pellets and succession during fecal pellet degradation. Journal of Plankton Research. 18, 257-273.

Hara, T.J., 1993. Role of olfaction in fish behaviour. in: Pitcher, T.J. (Ed.), The behaviour of teleost fishes. Chapman & Hall, London, pp. 152-176.

Heath, P., Moore, C., 1997. Rearing dover sole larvae on *Tisbe* and *Artemia* diets. Aquaculture International. 5, 29-39.

Heip, C., Smol, N., 1976. Influence of temperature on the reproductive potential of two brackish-water harpacticoids (Crustacea: Copepoda). Marine Biology. 35, 327-334.

Hicks, G.R.F., 1980. Structure of phytal harpacticoid copepod assemblages and the influence of habitat complexity and turbidity. Journal of Experimental Marine Biology and Ecology. 44, 157-192.

Hicks, G.R.F., 1982. Habitat structure, disturbance, and equilibrium in crustacean communities. Marine Ecology. 3, 41-51.

Hicks, G.R.F., Coull, B.C., 1983. The ecology of marine meiobenthic harpacticoid copepods. Oceanography and marine biology: an annual review. 21, 67-175.

Houde, E., 2002. Mortality. in: Fuiman, L.A., Werner, R.G. (Eds.), Fishery science: the unique contributions of early life stages. Blackwell Science, Oxford, pp. 64-87.

Hunt von Herbing, I., 2001. Development of feeding structures in larval fish with different life histories: winter flounder and Atlantic cod. Journal of Fish Biology. 59, 767-782.

Hunt von Herbing, I., Gallager, S., 2000. Foraging behavior in early Atlantic cod larvae (*Gadus morhua*) feeding on a protozoan (*Balanion* sp.) and a copepod nauplius (*Pseudodiaptomus* sp.). Marine Biology. 136, 591-602.

Hunt von Herbing, I., Gallager, S.M., Halteman, W., 2001. Metabolic costs of pursuit and attack in early larval Atlantic cod. Marine Ecology Progress Series. 216, 201-212.

Hunter, J.R., 1972. Swimming and feeding behavior of larval anchovy *Engraulis mordax*. Fishery Bulletin. 70, l-834.

Hunter, J.R., 1980. The feeding behavior and ecology of marine fish larvae. in: Bardach, J.E., Magnuson, J.J., May, R.C., Reinhart, J.M. (Eds.), Fish behavior and its use in the capture and culture of fishes. ICLARM, Manila, pp. 287-326.

Huys, R., Boxshall, G.A., 1991. Copepod evolution. Ray Society London.

Hwang, J.-S., Turner, J.T., 1995. Behaviour of cyclopoid, harpacticoid, and calanoid copepods from coastal waters of Taiwan. Marine Ecology. 16, 207-216.

Ismar, S., Hansen, T., Sommer, U., 2008. Effect of food concentration and type of diet on *Acartia* survival and naupliar development. Marine Biology. 154, 335-343.

Izquierdo, M., Koven, W., 2011. Lipids. in: Holt, G.J. (Ed.), Larval Fish Nutrition. Wiley-Blackwell, Oxford, pp. 47-81.

Kahan, D., 1979. Vegetables as food for marine harpacticoid copepods. Aquaculture. 16, 345-350.

Kahan, D., Uhlig, G., Schwenzer, D., Horowitz, L., 1982. A simple method for cultivating harpacticoid copepods and offering them to fish larvae. Aquaculture. 26, 303-310.

Kiørboe, T., Visser, A., 1999. Predator and prey perception in copepods due to hydromechanical signals. Marine Ecology Progress Series. 179, 81-95.

Kiørboe, T., Munk, P., Støttrup, J.G., 1985. First feeding by larval herring *Clupea harengus* L. Dana. 5, 95-107.

Kiørboe, T., Saiz, E., Viitasalo, M., 1996. Prey switching behaviour in the planktonic copepod *Acartia tonsa*. Marine Ecology Progress Series. 143, 65-75.

Kjørsvik, E., Pittman, K., Pavlov, D., 2004. From fertilisation to the end of metamorphosis—functional development. in: Moksness, E., Kjørsvik, E., Olsen, Y. (Eds.), Culture of cold-water marine fish. Blackwell Publishing, Oxford, pp. 204-278.

Klein Breteler, W.C.M., Gonzalez, S.R., Schogt, N., 1995. Development of *Pseudocalanus elongatus* (Copepoda, Calanoida) cultured at different temperature and food conditions. Marine Ecology Progress Series. 119, 99-99.

Knuckey, R.M., Semmens, G.L., Mayer, R.J., Rimmer, M.A., 2005. Development of an optimal microalgal diet for the culture of the calanoid copepod *Acartia sinjiensis*: Effect of algal species and feed concentration on copepod development. Aquaculture. 249, 339-351.

References

Kolkovski, S., Koven, W., Tandler, A., 1997. The mode of action of *Artemia* in enhancing utilization of microdiet by gilthead seabream *Sparus aurata* larvae. Aquaculture. 155, 193-205.

Koumoundouros, G., Maingot, E., Divanach, P., Kentouri, M., 2002. Kyphosis in reared sea bass (*Dicentrarchus labrax* L.): ontogeny and effects on mortality. Aquaculture. 209, 49-58.

Krebs, J.M., Turingan, R.G., 2003. Intraspecific variation in gape–prey size relationships and feeding success during early ontogeny in red drum, *Sciaenops ocellatus*. Environmental Biology of Fishes. 66, 75-84.

Lang, I., Hodac, L., Friedl, T., Feussner, I., 2011. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology. 11, 124.

Léger, P., Bengtson, D., Simpson, K., Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. Oceanography and marine biology: an annual review. 24, 521-623.

Liddle, R.A., 2000. Regulation of cholecystokinin secretion in humans. Journal of gastroenterology. 35, 181-187.

Lubzens, E., Zmora, O., Barr, Y., 2001. Biotechnology and aquaculture of rotifers. Hydrobiologia. 446-447, 337-353.

MacKenzie, B.R., Kiørboe, T., 1995. Encounter rates and swimming behavior of pause-travel and cruise larval fish predators in calm and turbulent laboratory environments. Limnology and Oceanography, 1278-1289.

Mahjoub, M.-S., Kumar, R., Souissi, S., Schmitt, F.G., Hwang, J.-S., 2012. Turbulence effects on the feeding dynamics in European sea bass (*Dicentrarchus labrax*) larvae. Journal of Experimental Marine Biology and Ecology. 416–417, 61-67.

Malzahn, A., Clemmesen, C., Rosenthal, H., 2003. Temperature effects on growth and nucleic acids in laboratory-reared larval coregonid fish. Marine Ecology Progress Series. 259, 285-293.

Martin, J.W., 1991. Branchiopoda. in: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Volume 9. Crustacea. Wiley-Liss Inc., New York, pp. 25-224.

McEvoy, L.A., Naess, T., Bell, J.G., Lie, Ø., 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. Aquaculture. 163, 237-250.

McIntyre, A.D., 1969. Ecology of marine meiobenthos. Biological Reviews of the Cambridge Philosophical Society. 44, 245-288.

McKinnon, A.D., Duggan, S., Nichols, P.D., Rimmer, M.A., Semmens, G., Robino, B., 2003. The potential of tropical paracalanid copepods as live feeds in aquaculture. Aquaculture. 223, 89-106.

Miller, T., Crowder, L., Rice, J., Marschall, E., 1988. Larval size and recruitment mechanisms in fishes: Toward a conceptual framework. Canadian Journal of Fisheries and Aquatic Sciences. 45, 1657-1670.

Moretti, A., Fernandez-Criado, M.P., Cittolin, G., Guidastri, R., 1999. Manual on hatchery production of seabass and gilthead seabream, Vol. 1. FAO, Rome.

Munilla-Moran, R., Stark, J.R., Barbour, A., 1990. The role of exogenous enzymes in digestion in cultured turbot larvae (*Scophthalmus maximus* L.). Aquaculture. 88, 337-350.

Munk, P., Kiørboe, T., 1985. Feeding behaviour and swimming activity of larval herring (*Clupea harengus*) in relation to density of copepod nauplii. Marine Ecology Progress Series. 24, 15-21.

Nanton, D.A., Castell, J.D., 1999. The effects of temperature and dietary fatty acids on the fatty acid composition of harpacticoid copepods, for use as a live food for marine fish larvae. Aquaculture. 175, 167-181.

Noodt, W., 1957. Zur Ökologie der Harpacticoidea (Crust. Cop.) des Eulitorals der deutschen Meeresküste und der angrenzenden Brackgewässer. Zoomorphology. 46, 149-242.

Norsker, N.H., Støttrup, J.G., 1994. The importance of dietary HUFAs for fecundity and HUFA content in the harpacticoid, *Tisbe holothuriae* Humes. Aquaculture. 125, 155-166.

O'Brien, W.J., Browman, H.I., Evans, B.I., 1990. Search strategies of foraging animals. American Scientist. 78, 152-160.

Øie, G., Reitan, K.I., Olsen, Y., 1994. Comparison of rotifer culture quality with yeast plus oil and algal-based cultivation diets. Aquaculture International. 2, 225-238.

Olivotto, I., Avella, M.A., Sampaolesi, G., Piccinetti, C.C., Navarro Ruiz, P., Carnevali, O., 2008a. Breeding and rearing the longsnout seahorse *Hippocampus reidi*: Rearing and feeding studies. Aquaculture. 283, 92-96.

Olivotto, I., Capriotti, F., Buttino, I., Avella, A.M., Vitiello, V., Maradonna, F., Carnevali, O., 2008b. The use of harpacticoid copepods as live prey for *Amphiprion clarkii* larviculture: Effects on larval survival and growth. Aquaculture. 274, 347-352.

Outzen, H., Berglund, G.I., Smalås, A.O., Willassen, N.P., 1996. Temperature and pH sensitivity of trypsins from atlantic salmon (*Salmo salar*) in comparison with bovine and porcine trypsin. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 115, 33-45.

Paffenhöfer, G.A., Strickler, J.R., Alcaraz, M., 1982. Suspension-feeding by herbivorous calanoid copepods: A cinematographic study. Marine Biology. 67, 193-199.

Parrish, C.C., French, V.M., Whiticar, M.J., 2012. Lipid class and fatty acid composition of copepods (*Calanus finmarchicus*, *C. glacialis*, *Pseudocalanus* sp., *Tisbe furcata* and *Nitokra lacustris*) fed various combinations of autotrophic and heterotrophic protists. Journal of Plankton Research. 34, 356-375.

Patil, V., Källqvist, T., Olsen, E., Vogt, G., Gislerød, H.R., 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquaculture International. 15, 1-9.

Payne, M.F., Rippingale, R.J., 2000. Evaluation of diets for culture of the calanoid copepod *Gladioferens imparipes*. Aquaculture. 187, 85-96.

Payne, M.F., Rippingale, R.J., 2001. Intensive cultivation of the calanoid copepod *Gladioferens imparipes*. Aquaculture. 201, 329-342.

Payne, M.F., Rippingale, R.J., Cleary, J.J., 2001. Cultured copepods as food for West Australian dhufish (*Glaucosoma hebraicum*) and pink snapper (*Pagrus auratus*) larvae. Aquaculture. 194, 137-150.

Peck, M.A., Holste, L., 2006. Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): Optimizing intensive cultures. Aquaculture. 255, 341-350.

Pedersen, B.H., Andersen, K.P., 1992. Induction of trypsinogen secretion in herring larvae (*Clupea harengus*). Marine Biology. 112, 559-565.

Pedersen, B.H., Nilssen, E.M., Hjelmeland, K., 1987. Variations in the content of trypsin and trypsinogen in larval herring (*Clupea harengus*) digesting copepod nauplii. Marine Biology. 94, 171-181.

Penglase, S., Nordgreen, A., van der Meeren, T., Olsvik, P.A., Sæle, Ø., Sweetman, J.W., Baeverfjord, G., Helland, S., Hamre, K., 2010. Increasing the level of selenium in rotifers (*Brachionus plicatilis* 'Cayman') enhances the mRNA expression and activity of glutathione peroxidase in cod (*Gadus morhua* L.) larvae. Aquaculture. 306, 259-269.

Perlmutter, D.G., Meyer, J.L., 1991. The impact of a stream-dwelling harpacticoid copepod upon detritally associated bacteria. Ecology, 2170-2180.

Planas, M., Pérez-Lorenzo, M., Hjelm, M., Gram, L., Uglenes Fiksdal, I., Bergh, Ø., Pintado, J., 2006. Probiotic effect in vivo of *Roseobacter* strain 27-4 against *Vibrio* (*Listonella*) *anguillarum* infections in turbot (*Scophthalmus maximus* L.) larvae. Aquaculture. 255, 323-333.

Poinar Jr., G.O., 2001. Nematoda and Nematomorpha. in: Thorp, J., Covich, A. (Eds.), Ecology and classification of north american freshwater invertebrates. Academic Press, New York, pp. 255-296.

References

Ponis, E., Probert, I., Véron, B., Le Coz, J.R., Mathieu, M., Robert, R., 2006. Nutritional value of six Pavlovophyceae for *Crassostrea gigas* and *Pecten maximus* larvae. Aquaculture. 254, 544-553.

Provasoli, L., 1968. Media and prospects for the cultivation of marine algae. in: Watanabe, A., Hattori, A. (Eds.), Cultures and Collections of Algae. Jpn. Soc. Plant Physiol., Hakone, pp. 63-75.

Provasoli, L., Shiraishi, K., Lance, J.R., 1959. Nutritional idiosyncrasies of *Artemia* and *Tigriopus* in monoxenic culture. Annals of the New York Academy of Sciences. 77, 250-261.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture. 155, 103-115.

Reitan, K.I., Rainuzzo, J.R., Olsen, Y., 1994. Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. Aquaculture International. 2, 33-48.

Renaud, S.M., Thinh, L.V., Parry, D.L., 1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture. 170, 147-159.

Rhodes, A., 2003. Methods for high density batch culture of *Nitokra lacustris*, a marine harpacticoid copepod. in: Browman, A.I., Shiftesvik, A.B. (Eds.), The Big Fish Bang, 26th Annual larval fish conference. Institute of Marine Research, Bergen, Norway, pp. 450-465.

Ribeiro, A.R.A., Ribeiro, L., Sæle, Ø., Hamre, K., Dinis, M.T., Moren, M., 2011. Iodine-enriched rotifers and *Artemia* prevent goitre in Senegalese sole (*Solea senegalensis*) larvae reared in a recirculation system. Aquaculture Nutrition. 17, 248-257.

Ribeiro, L., Zambonino-Infante, J.L., Cahu, C., Dinis, M.T., 1999. Development of digestive enzymes in larvae of *Solea senegalensis*, Kaup 1858. Aquaculture. 179, 465-473.

Rodríguez, C., Pérez, J.A., Badía, P., Izquierdo, M.S., Fernández-Palacios, H., Hernández, A.L., 1998. The n-3 highly unsaturated fatty acids requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in the diet. Aquaculture. 169, 9-23.

Rønnestad, I., Morais, S., 2008. Digestion. in: Finn, R.N., Kapoor, B.G. (Eds.), Fish larval physiology. Science Publishers, Enfield, pp. 201-262.

Rønnestad, I., Helland, S., Lie, Ø., 1998. Feeding *Artemia* to larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) results in lower larval vitamin A content compared with feeding copepods. Aquaculture. 165, 159-164.

Rønnestad, I., Kamisaka, Y., Conceição, L.E.C., Morais, S., Tonheim, S.K., 2007. Digestive physiology of marine fish larvae: Hormonal control and processing capacity for proteins, peptides and amino acids. Aquaculture. 268, 82-97.

Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., Boglione, C., 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. Reviews in Aquaculture. doi: 10.1111/raq.12010.

Rønnestad, I., Tonheim, S., Fyhn, H., Rojas-Garcıa, C., Kamisaka, Y., Koven, W., Finn, R., Terjesen, B., Barr, Y., Conceição, L., 2003. The supply of amino acids during early feeding stages of marine fish larvae: a review of recent findings. Aquaculture. 227, 147-164.

Roo, F.J., Hernández-Cruz, C.M., Socorro, J.A., Fernández-Palacios, H., Montero, D., Izquierdo, M.S., 2009. Effect of DHA content in rotifers on the occurrence of skeletal deformities in red porgy *Pagrus pagrus* (Linnaeus, 1758). Aquaculture. 287, 84-93.

Rosenthal, H., 1968. Schwimmverhalten und Schwimmgeschwindigkeit bei den Larven des Herings *Clupea harengus*. Helgoland Marine Research. 18, 453-486.

Rosenthal, H., 1969. Untersuchungen über das Beutefangverhalten bei Larven des Herings *Clupea harengus*. Marine Biology. 3, 208-221.

Rosenthal, H., Hempel, G., 1970. Experimental studies in feeding and food requirements of herring larvae (*Clupea harengus* L.). in: Steele, J.H. (Ed.), Marine food chains. University of California Press, Berkeley and Los Angeles, pp. 344–364.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999a. Recent developments in the essential fatty acid nutrition of fish. Aquaculture. 177, 191-199.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999b. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture. 179, 217-229.

Sargent, J.R., McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture. 155, 117-127.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. Journal of Applied Ichthyology. 11, 183-198.

Schipp, G., 2006. The use of calanoid copepods in semi-intensive, tropical marine fish larviculture. in: Cruz-Suarez, E., Ricque Marie, D., Salazar, M., López, M., Cavazos, D., Puello Cruz, A., Garcia Ortega, A. (Eds.), Avances em Nutrición Acuícola VIII. VIII Simposium Internacional de Nutrición Acuícola, Mexico, pp. 15-17.

Seifried, S., Dürbaum, J., 2000. First clear case of carnivory in marine Copepoda Harpacticoida. Journal of Natural History. 34, 1595-1618.

Shields, R.J., Bell, J.G., Luizi, F.S., Gara, B., Bromage, N.R., Sargent, J.R., 1999. Natural copepods are superior to enriched *Artemia* nauplii as feed for Halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. The Journal of Nutrition. 129, 1186-1194.

Sibert, J., Brown, T.J., Healey, M.C., Kask, B.A., Naiman, R.J., 1977. Detritus-based food webs: Exploitation by juvenile chum salmon (*Oncorhynchus keta*). Science. 196, 649-650.

Smol, N., Heip, C., 1974. The culturing of some harpacticoid copepods from brackish water. Biologisch Jaarboek (Dodonaea). 42.

Southgate, P.C., 2003. Feeds and feed production. in: Lucas, J.S., Southgate, P.C. (Eds.), Aquaculture: farming aquatic animals and plants. Blackwell Publishing, Oxford, pp. 172-198.

Sprecher, H., 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 1486, 219-231.

Statistisches-Bundesamt, 2012. Aquakultur 2011: 39000 Tonnen Fische und Muscheln in Deutschland erzeugt, Wiesbaden.

Støttrup, J., Norsker, N., 1997. Production and use of copepods in marine fish larviculture. Aquaculture. 155, 231-247.

Støttrup, J.G., 2000. The elusive copepods: their production and suitability in marine aquaculture. Aquaculture Research. 31, 703-711.

Støttrup, J.G., Richardson, K., Kirkegaard, E., Pihl, N.J., 1986. The cultivation of *Acartia tonsa* Dana for use as a live food source for marine fish larvae. Aquaculture. 52, 87-96.

Strickler, J.R., Balázsi, G., 2007. Planktonic copepods reacting selectively to hydrodynamic disturbances. Philosophical Transactions of the Royal Society B: Biological Sciences. 362, 1947-1958.

Theilacker, G.H., Kimball, A., 1984. Comparative quality of rotifers and copepods as foods for larval fishes. California Cooperative Oceanic Fisheries Investigations Reports. 25, 80-85.

Thetmeyer, H., Kils, U., 1995. To see and not be seen: the visibility of predator and prey with respect to feeding behaviour. Marine Ecology Progress Series. 126, 1-8.

Titelman, J., 2001. Swimming and escape behavior of copepod nauplii: implications for predator-prey interactions among copepods. Marine Ecology Progress Series. 213, 203-213.

Titelman, J., Kiørboe, T., 2003. Predator avoidance by nauplii. Marine Ecology Progress Series. 247, 137-149.

Tocher, D., 2009. Issues surrounding fish as a source of omega-3 long-chain polyunsaturated fatty acids. Lipid Technology. 21, 13-16.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science. 11, 107-184.

References

Tocher, D.R., Sargent, J.R., 1984. Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. Lipids. 19, 492-499.

Tocher, D.R., Bendiksen, E.Å., Campbell, P.J., Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture. 280, 21-34.

Turingan, R.G., Beck, J.L., Krebs, J.M., Licamele, J.D., 2005. Development of feeding mechanics in marine fish larvae and the swimming behavior of zooplankton prey: implications for rearing marine fishes. in: Lee, C.-S., O'Bryen, P.J., Marcus, N.H. (Eds.), Copepods in Aquaculture. Blackwell Publishing, Ames, USA, pp. 119-132.

Turner, J.T., 1984. The feeding ecology of some zooplankters that are important prey items of larval fish. NOAA Technical Report NMFS. 7, 1-28.

Turner, J.T., 2004. The importance of small planktonic copepods and their roles in pelagic marine food webs. Zoological Studies. 43, 255-266.

Ueberschär, B., 1995. The use of tryptic enzyme activity measurement as a nutritional condition index: laboratory calibration data and field application, ICES Mar Sci Symp, pp. 119-129.

Ueberschär, B., 2006. Critical times for fish larvae - Measurement of tryptic activity in order to assess the nutritional condition of fish larvae from laboratory rearing and from field samples - an overview. Verhandlungen der Gesellschaft für Ichthyologie. 5, 193-229.

Ueberschär, B., Clemmesen, C., 1992. A comparison of the nutritional condition of herring larvae as determined by two biochemical methods - tryptic enzyme activity and RNA/DNA ratio measurements. ICES Journal of Marine Science: Journal du Conseil. 49, 245-249.

Uhlig, G., 1984. Progress in mass cultivation of harpacticoid copepods for mariculture purposes. Special Publication of the European Mariculture Society. 8, 261-273.

van der Meeren, T., Olsen, R.E., Hamre, K., Fyhn, H.J., 2008. Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. Aquaculture. 274, 375-397.

Veloza, A.J., Chu, F.-L.E., Tang, K.W., 2006. Trophic modification of essential fatty acids by heterotrophic protists and its effects on the fatty acid composition of the copepod *Acartia tonsa*. Marine Biology. 148, 779-788.

Viitasalo, M., Kiørboe, T., Flinkman, J., Pedersen, L.W., Visser, A., 1998. Predation vulnerability of planktonic copepods: consequences of predator foraging strategies and prey sensory abilities. Marine Ecology Progress Series. 175, 129-142.

Villamil, L., Figueras, A., Planas, M., Novoa, B., 2003. Control of *Vibrio alginolyticus* in *Artemia* culture by treatment with bacterial probiotics. Aquaculture. 219, 43-56.

Volkman, J.K., Dunstan, G.A., Jeffrey, S.W., Kearney, P.S., 1991. Fatty acids from microalgae of the genus *Pavlova*. Phytochemistry. 30, 1855-1859.

Waggett, R., Buskey, E., 2007. Calanoid copepod escape behavior in response to a visual predator. Marine Biology. 150, 599-607.

Walford, J., Lam, T.J., 1993. Development of digestive tract and proteolytic enzyme activity in seabass (*Lates calcarifer*) larvae and juveniles. Aquaculture. 109, 187-205.

Wang, C., Lovell, R.T., 1997. Organic selenium sources, selenomethionine and selenoyeast, have higher bioavailability than an inorganic selenium source, sodium selenite, in diets for channel catfish (*Ictalurus punctatus*). Aquaculture. 152, 223-234.

Watson, B., 1965. The fine structure of the body-wall in a free-living nematode, *Euchromadora vulgaris*. Quarterly Journal of Microscopical Science. 3, 75-81.

Wijkstrom, U., 2003. Short and long-term prospects for consumption of fish. Veterinary Research Communications. 27, 461-468.

Wright, K.A., 1991. Nematoda. in: Harrison, F.W., Ruppert, E.E. (Eds.), Microscopic anatomy of invertebrates: Volume 4. Aschelminthes. Wiley-Liss Inc., New York, pp. 111-195.

Wynn, J., 2011. Lipids, Fatty Acids. in: Murray, M.-Y. (Ed.), Comprehensive Biotechnology. Academic Press, Burlington, pp. 41-52.

Yúfera, M., 2011. Feeding behavior in Larval Fish. in: Holt, G.J. (Ed.), Larval Fish Nutrition. Wiley-Blackwell, Oxford, pp. 283-305.

Zambonino Infante, J.L., Cahu, C., 1994. Development and response to a diet change of some digestive enzymes in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiology and Biochemistry. 12, 399-408.

Zambonino Infante, J.L., Cahu, C.L., 1999. High dietary lipid levels enhance digestive tract maturation and improve *Dicentrarchus labrax* larval development. The Journal of Nutrition. 129, 1195-1200.

Zambonino Infante, J.L., Cahu, C.L., 2001. Ontogeny of the gastrointestinal tract of marine fish larvae. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 130, 477-487.

Zavala-Leal, I., Dumas, S., Peña, R., Contreras-Olguín, M., Hernández-Ceballos, D., 2012. Effects of culture conditions on feeding response of larval Pacific red snapper (*Lutjanus peru*, Nichols & Murphy) at first feeding. Aquaculture Research. doi: 10.1111/j.1365-2109.2012.03144.x.

Zhang, Q., Uhlig, G., 1993. Effect of density on larval development and female productivity of *Tisbe holothuriae* (Copepoda, Harpacticoida) under laboratory conditions. Helgoland Marine Research. 47, 229-241.

Zhukova, N.V., Aizdaicher, N.A., 1995. Fatty acid composition of 15 species of marine microalgae. Phytochemistry. 39, 351-356.

Zurlini, G., Ferrari, I., Nassogne, A., 1978. Reproduction and growth of *Euterpina acutifrons* (Copepoda: Harpacticoida) under experimental conditions. Marine Biology. 46, 59-64.

Appendix

Table A1: Fatty acid composition (ng µg C^{-1}) of five different algal species, which were fed to *Tisbe sp.*

Rhodo = *Rhodomonas sp.*, Phaeo = *Phaeodactylum tricornutum*, Pav = *Pavlova sp.*, Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana*. Values are mean ± standard deviation of the mean (N = 3), (nd = non detectable level).

	Rhodo	Phaeo	Pav	Duna	Iso
C14:0	2.8 ± 0.4	8.1 ± 0.3	6.5 ± 0.5	0.3 ± 0.5	32.2 ± 8.2
C16:0	25.2 ± 9.2	29.3 ± 4.1	52.4 ± 13.2	29.4 ± 3.4	30.3 ± 3.3
C16:1ω7+ C *iso*-17:0	nd	19.0 ± 1.7	23.9 ± 2.9	2.4 ± 0.2	3.0 ± 0.6
C16:4ω3	nd	nd	nd	20.8 ± 2.1	nd
C18:0	12.5 ± 5.7	14.9 ± 4.3	20.0 ± 5.9	5.1 ± 1.2	7.2 ± 1.2
C18:1ω9c	2.8 ± 0.6	1.3 ± 0.3	8.0 ± 1.0	4.5 ± 0.6	28.3 ± 2.4
C18:1ω7	13.9 ± 6.2	1.3 ± 0.2	nd	1.3 ± 0.3	2.1 ± 0.7
C18:2ω6c	3.1 ± 0.4	1.4 ± 0.3	4.2 ± 0.7	5.4 ± 0.7	11.4 ± 0.9
C18:3ω6	nd	nd	nd	6.5 ± 0.9	nd
C18:3ω3	11.2 ± 2.7	nd	1.3 ± 0.95	46.4 ± 5.8	7.5 ± 2.4
C18:4ω3	17.5 ± 4.2	nd	nd	1.8 ± 0.1	12.2 ± 1.7
C20:4ω6	0.44 ± 0.07	0.76 ± 0.14	11.6 ± 0.8	nd	0.43 ± 0.06
C20:3ω3	nd	nd	nd	nd	nd
C20:5ω3	11.2 ± 1.9	51.6 ± 4.0	42.9 ± 2.6	nd	0.63 ± 0.21
C22:6ω3	7.5 ± 1.1	2.1 ± 0.6	1.2 ± 0.5	nd	16.6 ± 6.5
total	111.0 ± 34.6	143.7 ± 3.3	183.7 ± 28.6	124.5 ± 13.9	139.5 ± 22.8
SFA[1]	42.7 ± 18.5	53.1 ± 8.3	81.1 ± 19.7	35.0 ± 3.6	70.4 ± 11.8
MUFA[2]	2.8 ± 0.6	33.4 ± 2.1	31.9 ± 3.8	6.9 ± 0.7	33.3 ± 1.3
PUFA[3]	33.5 ± 6.1	55.9 ± 4.8	62.5 ± 5.2	58.3 ± 7.3	36.8 ± 10.1
DHA:EPA	0.67 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	-	26.3 ± 2.0
EPA:ARA	25.7 ± 5.1	68.5 ± 7.0	3.7 ± 0.04	-	1.5 ± 0.5
ω6	4.1 ± 0.6	2.1 ± 0.4	17.0 ± 1.9	12.3 ± 2.2	11.9 ± 1.0
ω3	47.5 ± 9.9	53.7 ± 4.5	45.4 ± 3.8	69.0 ± 7.9	37.1 ± 10.8
ω3:ω6	11.6 ± 0.9	25.4 ± 2.4	2.7 ± 0.3	5.6 ± 0.4	3.1 ± 0.7

[1] Saturated fatty acids include additionally 12:0, 20:0, 22:0 and 24:0.
[2] Monounsaturated fatty acids include additionally 14:1, 15:1, 17:1, 18:1ω9t, 20:1ω9, 22:1ω9 and 24:1.
[3] Polyunsaturated fatty acids include additionally 16:2, 16:3, 16:4ω3, 18:2ω6t, 20:2ω6, 20:3ω6 and 22:2.

Table A2: Fatty acid composition (ng µg C^{-1}) of five different algal species, which were fed to *Tachidius discipes.*

Rhodo = *Rhodomonas sp.*, Phaeo = *Phaeodactylum tricornutum*, Pav = *Pavlova sp.*, Duna = *Dunaliella tertiolecta*, Iso = *Isochrysis galbana*. Values are mean ± standard deviation of the mean (N = 3), (nd = non detectable level).

	Rhodo	Phaeo	Pav	Duna	Iso
C14:0	11.1 ± 1.4	23.1 ± 4.1	6.5 ± 0.5	0.83 ± 0.08	84.0 ± 6.7
C16:0	40.6 ± 8.1	49.6 ± 14.3	52.4 ± 13.2	25.1 ± 4.6	61.4 ± 7.3
C16:1ω7+ C *iso*-17:0	3.9 ± 1.6	70.6 ± 22.3	23.9 ± 2.9	2.1 ± 0.5	4.9 ± 0.3
C16:4ω3	nd	nd	nd	16.1 ± 1.8	nd
C18:0	7.9 ± 2.2	2.9 ± 1.9	20.0 ± 5.9	9.5 ± 1.3	5.6 ± 1.1
C18:1ω9c	7.6 ± 4.6	5.3 ± 3.3	8.0 ± 1.0	3.9 ± 0.4	74.8 ± 10.4
C18:1ω7	15.4 ± 0.8	1.7 ± 1.4	nd	1.8 ± 0.4	8.5 ± 0.6
C18:2ω6c	11.1 ± 3.4	5.6 ± 0.6	4.2 ± 0.7	5.1 ± 0.8	20.3 ± 2.1
C18:3ω6	1.7 ± 0.7	2.4 ± 0.8	nd	6.2 ± 0.4	0.78 ± 0.10
C18:3ω3	56.8 ± 6.1	0.42 ± 0.39	1.33 ± 0.95	35.4 ± 5.4	18.0 ± 1.9
C18:4ω3	48.3 ± 8.8	2.4 ± 2.0	nd	2.5 ± 0.3	25.1 ± 4.9
C20:4ω6	1.1 ± 0.7	1.8 ± 0.3	11.6 ± 0.8	nd	nd
C20:3ω3	nd	nd	nd	0.60 ± 0.17	nd
C20:5ω3	25.1 ± 1.6	74.8 ± 6.4	42.7 ± 2.6	nd	1.3 ± 0.2
C22:6ω3	17.1 ± 0.8	6.8 ± 1.1	1.2 ± 0.5	0.30 ± 0.13	22.7 ± 1.7
total	252.8 ± 30.4	276.9 ± 51.9	183.7 ± 28.6	115.5 ± 15.6	336.2 ± 32.9
SFA[1]	60.1 ± 11.5	78.9 ± 19.7	81.1 ± 19.7	35.8 ± 5.1	152.5 ± 15.0
MUFA[2]	30.9 ± 6.6	79.9 ± 26.9	31.9 ± 3.8	9.7 ± 1.5	92.0 ± 10.3
PUFA[3]	162.2 ± 16.3	118.7 ± 10.9	62.5 ± 5.2	70.0 ± 9.3	91.7 ± 8.6
DHA:EPA	0.68 ± 0.02	0.09 ± 0.01	0.03 ± 0.01	-	18.5 ± 3.7
EPA:ARA	32.7 ± 25.0	42.1 ± 7.7	3.69 ± 0.04	-	-
ω6	14.3 ± 4.3	9.7 ± 1.1	17.0 ± 1.9	11.7 ± 1.4	21.8 ± 2.2
ω3	147.3 ± 17.2	84.4 ± 9.4	45.4 ± 3.8	55.0 ± 7.5	67.1 ± 6.4
ω3:ω6	11.2 ± 4.9	8.7 ± 0.7	2.7 ± 0.3	4.7 ± 0.2	3.1 ± 0.1

[1] Saturated fatty acids include additionally 12:0, 20:0, 22:0 and 24:0.

[2] Monounsaturated fatty acids include additionally 14:1, 15:1, 17:1, 18:1ω9t, 20:1ω9, 22:1ω9 and 24:1.

[3] Polyunsaturated fatty acids include additionally 16:2, 16:3, 16:4ω3, 18:2ω6t, 20:2ω6, 20:3ω6 and 22:2.

Contribution of authors

The work presented in this thesis was conducted within the NEMO-project TP3 entitled „Rearing of harpacticoid copepods as food for marine fish larvae" coordinated by Prof. Dr. Ulrich Sommer.

Chapter 1: Effect of algal species and concentration on development and fatty acid composition of two harpacticoid copepods, *Tisbe* sp. and *Tachidius discipes*, and a discussion about their suitability for marine fish larvae
Carmen Arndt (CA), Ulrich Sommer (US)
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Planning of experiments: CA and US. Conduction of experiments and analysis of the parameters: CA. Writing: CA, with assistance of US.

Chapter 2: Evaluation of the suitability of benthic copepods as food for fish larvae and the potential digestibility of prey
Carmen Arndt (CA), Ulrich Sommer (US), Bernd Ueberschär (BU)

Planning of experiments: CA and BU. Conduction of the experiments and analysis of the parameters: CA. Writing: CA, with helpful comments of BU and US.

Chapter 3: Providing harpacticoid copepods via swimming sieve improves fish larval feeding success
Carmen Arndt (CA), Maud Moison (MM), Ulrich Sommer (US)
Submitted to Aquaculture

Planning of the experiment: CA. Conduction of the experiment: CA and MM. Extraction of the trajectories: CA and MM. MM provided the Matlab-program with which the extracted trajectories could be analysed. Analysis of the parameters: CA. Writing: CA, with helpful comments of MM and US.

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Publikationen

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Arndt, C., Moison, M., Sommer, U., (subm.) Providing harpacticoid copepods via floating sieve improves fish larval feeding success. *Submitted to Aquaculture*

Eidesstattliche Erklärung

Hiermit erkläre ich, dass die vorliegende Promotionsarbeit selbständig von mir angefertigt wurde. Die Dissertation ist in Form und Inhalt meine eigene Arbeit und es wurden keine anderen als die angegebenen Hilfsmittel verwendet. Diese Arbeit wurde weder ganz noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Teile dieser Arbeit wurden zur Begutachtung in Fachzeitschriften eingereicht. Die Dissertation ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden. Dies ist mein erstes Promotionsverfahren.

Kiel, den 31.01.2013

Carmen Arndt